Science
Library

OCT 19 1942

Volume 32 133 *Number 10*

Phytopathology

AN INTERNATIONAL JOURNAL

October 1942

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter

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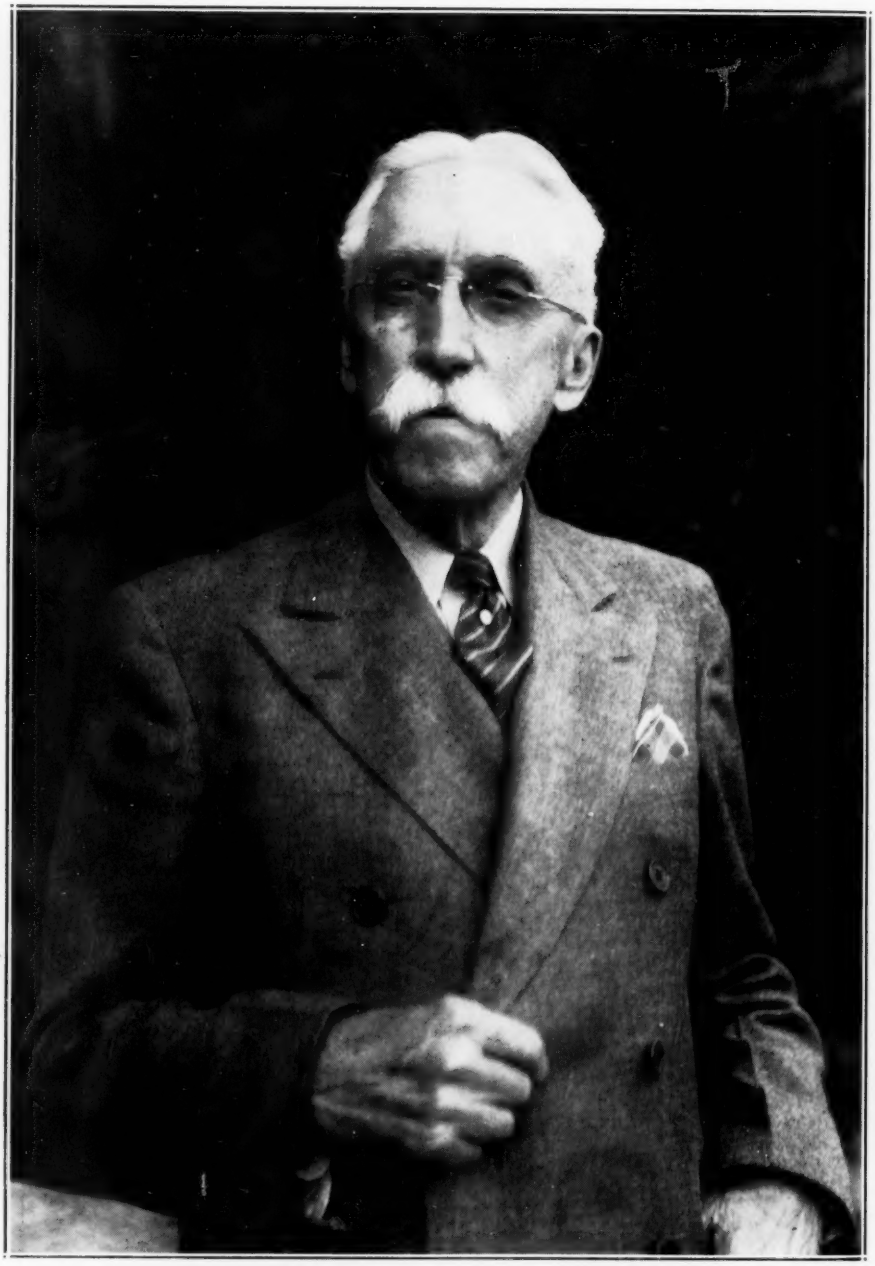

JOSEPH CHARLES ARTHUR

JOSEPH CHARLES ARTHUR
1850–1942

FRANK D. KERN

In the death of Joseph Charles Arthur at Brook, Indiana, April 30, 1942, we have lost an eminent botanist, a pioneer plant pathologist, and one of the foremost students of plant rusts in the world.

Joseph Charles Arthur was born at Lowville, New York, January 11, 1850, the son of Charles and Ann (Allen) Arthur. His earliest paternal American ancestor was his great-great-grandfather who came from England about 1745 and settled in Groton, Connecticut. The first American-born ancestor was Richard Arthur, whose wife, Hannah Bradford, was a great-grand-daughter of Governor William Bradford, one of the Mayflower pilgrims. One of the sons of this marriage was Joseph Arthur, grandfather of Joseph Charles Arthur. After the death of her husband Mrs. Richard Arthur moved from Connecticut to northern New York. This was the family abode for a considerable time. However, Charles Arthur, father of Joseph Charles, went westward when his son was about six years old and settled near Sterling, Illinois, but soon pushed on into Iowa. The family home was at Charles City, Iowa, for a number of years, and then at Spirit Lake. At Charles City they lived for a time in town and later on a farm.

Joseph Charles received his preliminary education in the country schools of Floyd County and in the high school of Charles City. The boy was christened Charles Joseph, but, since his father's name was Charles, he was called Joseph, at home. In school he was registered as C. Joseph Arthur. When the teacher called the roll the other youngsters often turned about in their seats to "see" Joseph Arthur. This was so annoying to the sensitive boy that he changed the order of his name to Joseph Charles and retained that form ever afterwards.

From Floyd County he went as a student to the newly established Iowa State College at Ames. Here, in 1872, he was graduated first on the list (alphabetically) of the first graduating class, with the degree of Bachelor of Science. In 1877 he earned there the degree of Master of Science. In 1886 he was the recipient of the first degree of Doctor of Science conferred by Cornell University in the field of plant pathology and mycology. His honorary degrees include the Doctor of Laws, University of Iowa, 1916; Doctor of Science from his alma mater in 1920; and Doctor of Science, Purdue University, 1931. He was a graduate student at Johns Hopkins and at Harvard in 1879 and at Bonn in 1896.

The ambition to be a botanist appeared very early in the life of Joseph Charles Arthur. He had it as a boy before he went to college. The development of this ambition and the persistence with which he clung to it make a most interesting story. How eminently well he succeeded has long been recognized in scientific circles everywhere. The early difficulties are worthy

of recounting because they illustrate the possibilities of accomplishment when a goal is set and persistently sought, in season and out, regardless of obstacles or discouragements. The subject of our sketch early showed two outstanding characteristics—he was a nonconformist to many rules and customs and he had a burning desire to get into botanical work. The first got him into some tight places in college. There were two college regulations with which he came into conflict—the one was that all students had to do manual labor on the college farm and the other was that all had to participate in military drill. He tried to circumvent both and succeeded fairly well. Assisting one of the professors in the laboratory got him out of the farm work and taking some broadsword exercises sufficed for the military drill. The combination of his disregard for regulations and his love for botany operated together to get him into a difficulty that nearly prevented his graduation. In 1872 the American Association for the Advancement of Science met at Dubuque, Iowa. The great botanist, Asa Gray, was to be a speaker. Of course, a budding botanical student desired to be there. In fact, he just had to go. But the college authorities did not think it wise and would not give their approval to his petition to be absent long enough to attend the meeting. No amount of persuasion changed their verdict. What did our independent, ambitious student do? He went. And he graduated a little later, but many amends had to be made.

His desire to start on a career in botany, however, was not so easily satisfied. His first disappointment was that botany was not being taught when he arrived at the Iowa State College. Before going to college he had found a copy of *The American Agriculturist* in which the parts of a flower were illustrated and described. He collected the plants of his home region, analyzed the flowers, and sought names for them. Although no one taught botany when he arrived as a freshman in college, the professor of agriculture helped him by supplying the first botanical book he had ever seen. It was Eton's Manual of about 1840. But the first real help came during his sophomore year when Dr. Charles E. Bessey arrived at Ames as a member of the instructional staff in 1870. An immediate friendship developed between instructor and student. Professor Bessey must have been delighted to have such an enthusiastic student of botany and the student was certainly fortunate to have contact with such a gifted and inspiring teacher. In later years Professor Bessey referred to Dr. Arthur as his first "botanical son" and was justly proud of him.

The college course then made a fine start toward specialization in botany, thanks to Professor Bessey. There were courses in vegetable physiology and economic plants, and lectures on weeds and parasitic fungi. Dr. Bessey's description of the last-named course was that the subject was illustrated by means of a good microscope, the College Herbarium, and a large collection of fungous plants. Little wonder that a student of the proclivity of Joseph Charles Arthur laid there the foundation for a long career in mycology. But, once through college, the young graduate was severely

tested. The modern era of botanical teaching and research was in its infancy. In 1872 botanical positions had not come into being in the colleges and universities. Agricultural experiment stations had not been founded, and there were no state or national departments of agriculture. Not until 1879, when he went to the University of Wisconsin as instructor in botany, did young Arthur succeed in obtaining a botanical position. But in the intervening years he never for a moment gave up the determination to make botany his life work, although he had to give attention to other things. He taught country schools several winters. He returned to the college at Ames several times to carry on special work. He prepared an exhibit for the Philadelphia Centennial Exposition in 1876. This consisted of a display of the flora of Iowa. For this he was awarded two diplomas and a bronze medal. In Philadelphia he worked for a time for a mineralogist. Then, going back to Iowa State, he became an assistant—not in botany but in zoology. He served also as an assistant in the library, where he helped to inaugurate the Dewey system of classification. His graduate work was in botany, the thesis being on the anatomy of *Echinocystis lobata* (wild cucumber-vine), and resulted in his receiving in 1877 the first Master of Science degree conferred by the College. For a while after that he had no work, but his yearning for an opportunity to work with plants led him to correspond with Peter Henderson, famous florist and seedsman of New York. This resulted in an opportunity for him to work in the Henderson greenhouses. This was in 1879. But the plan was never carried out. On his way to New York he stopped off at Baltimore to explore the city and more particularly the newly established Johns Hopkins University. A more or less chance interview with the President resulted in the offer of a complimentary fellowship of the value of $100.00. The opportunity to stay for a term was particularly welcome because the renowned mycologist, Dr. William G. Farlow, of Harvard, was coming within a week to begin a course of lectures. The Peter Henderson plan was abandoned. This spring term at Johns Hopkins was followed immediately with a summer term at Harvard. Work with Dr. Farlow was continued and other work was taken with Dr. George Lincoln Goodale and Dr. Asa Gray. What more could a struggling botanical student have desired? But there was more—John M. Coulter and Charles R. Barnes were fellow students. Lasting associations dated from that summer. Two or three years later Arthur, Barnes, and Coulter were working on the Handbook of Plant Dissection, which naturally came to be known as the ABC book. Botanical science is also indebted to these same three men for the *Botanical Gazette*, which they jointly edited over a long period of years.

In 1884 Joseph Charles Arthur was elected botanist in the newly founded Agricultural Experiment Station at Geneva. Here was a position for which he was well prepared and to which he was well suited. He had not been entirely happy in the teaching positions he had held just before at Wisconsin and Minnesota. The opportunity to do full-time research was exactly what

he had desired. He made good from the start. His early work at Geneva with pear blight is known to all plant pathologists. While there he took advantage of the opportunity to do graduate work at Cornell, which brought him a doctor's degree in 1886.

In 1887 Dr. Arthur was called to Purdue University as Professor of Botany. The next year his title was changed to Professor of Vegetable Physiology and Pathology and Botanist to the Indiana Agricultural Experiment Station. This position he held until his formal retirement in 1915. From then until his death he was Emeritus Professor of Botany at Purdue. He retained his residence in Lafayette and continued his work at the University for many years. He was married to Emily Stiles Potter, of Lafayette, Indiana, in 1901, also a person of New England extraction. Mrs. Arthur died in 1935.

During the earlier years of his connection at Purdue Dr. Arthur taught plant physiology and plant pathology. He was much interested in designing and building apparatus for laboratory work in plant physiology, but he spent most of his time on research in plant pathology and mycology. His work on the cereal smuts and on potato scab was important scientifically and valuable economically. He was the first to use formaldehyde for the prevention of potato scab.

Although an important and pioneer contributor to other botanical fields, particularly physiology and pathology, Dr. Arthur must be most praised for his work with that intriguing group of fungi known as the plant rusts. For many years he has been recognized as a world authority on this group of fungi. His interest in the rusts began while he was about the college at Ames in the years following his graduation. Dr. Bessey had purchased a collection from the Curtis Herbarium. In it were many specimens named by M. J. Berkeley but there were also specimens which were unnamed. To the young investigator it seemed that names must be supplied. Then began the describing of new species in his first rust paper on "Iowa Uromyces" published in 1882. From then until the publication of his last paper in 1936 he was continuously investigating the rusts, their life-cycles, genetic relationships, and geographical distribution. In 1899 he began a series of culture studies which continued for nineteen years. The life-histories and host relationships of many species, possibly a hundred or more, were revealed by these painstaking experiments. In summarizing this work (see Memoranda and Index of Cultures of Uredineae, 1899–1917, Mycologia **13**: 230–262, 1921) Dr. Arthur states that more than 2390 collections were used and that about 3750 sowings were made. When it is realized that almost every culture involved a potted plant growing in a greenhouse some idea of the magnitude of the work may be gained. Almost more phenomenal is the fact that more than 85 botanical correspondents contributed specimens and field observations year after year. A large number of these correspondents were not professional botanists and many of them acquired their interest, ability, and inclination either through personal contacts or correspondence with

Dr. Arthur. It is a marvelous example of how invaluable voluntary assistance may be enlisted through boundless enthusiasm and fine inspiration. Throughout his long professional career Dr. Arthur's work was never limited to the facilities and resources of the institution with which he was connected but extended far beyond that range.

In 1905 he brought out a new classification for the order Uredinales. Although he modified this classification in later years it served in its time as a great impetus to the researches on the group. In 1907 he began the preparation of a taxonomic treatment of the North American Uredinales for the *North American Flora* (published by the New York Botanical Garden). This ran into 11 parts consisting of a total of 765 pages and required 20 years for completion. Two books have been published—a volume titled *The Plant Rusts* in 1929 (in collaboration with F. D. Kern, C. R. Orton, F. D. Fromme, H. S. Jackson, E. B. Mains, and G. B. Bisby, all former associates in his laboratories), a biological treatment, and a *Manual of the Rusts in United States and Canada*, in 1934, a taxonomic treatment. In addition to these larger publications he found time to bring out a very long list of papers dealing with varied aspects of the rusts. Many collectors sent him their specimens. He determined and reported on collections not only from the United States but from Canada, Cuba, Puerto Rico, Mexico, Guatemala, South America, and the Philippines. He made collecting trips himself to the Rocky Mountains, New England, several southeastern States, and to Texas, New Mexico, and Arizona in the Southwest. He made numerous trips to Europe partly because of his fondness for travel but mostly to study type specimens in some old world Herbarium or Botanical Garden, to obtain access to some rare literature, or to confer with fellow workers, or to attend an International Botanical Congress where botanical nomenclature was under discussion. He was naturally much concerned with the rules of nomenclature, not only as they applied to his group of rusts, but also as they applied to the host plants. He was active in the Congresses at Vienna, 1905, Brussels, 1910, and Cambridge, 1930. In 1925 a trip was planned especially to confer with European mycologists who had special interests in the rusts. It was the privilege of the writer to accompany Dr. Arthur on this trip. Visits were made with Klebahn, Sydow, Dietel, Kniep, Lagerheim, Eriksson, Juel, Jørstad, Gäumann, Ramsbottom, Butler, and Miss Wakefield. A paper in *Science* (Vol. 43, pp. 558–560), entitled Conversations with European Mycologists, illustrates the value of exchanging opinions with fellow botanists in Germany, Sweden, Norway, Switzerland, and England. It was a strong link in a chain of cosmopolitan activities forged by Dr. Arthur throughout his long life.

It is difficult to render an adequate appraisal of Dr. Arthur's contributions to our knowledge of the plant rusts. They are set forth in 150 papers over a period of more than fifty years. The total number of papers on all subjects is 289. No detail was ever too small to be considered and no effort was too great if there was a possibility of arriving at a clearer understanding

of the identity and relationships of the various forms and species. Nor can we measure the results merely by reviewing his own publications but must take into consideration how he reached out into the lives of his students, his assistants, his correspondents, and his colleagues, spurring them on to an ever widening circle of activities. Some of his associates, who in their own turn have been active, have already been named as collaborators in the book on The Plant Rusts. We would fail if we did not name Bolley, Olive, Whetzel, Johnson (A. G.), Christman, Dodge, Garrett, Bethel, Holway, Rosen, Stevens (F. L.), Clinton, Kunkel, Thurston, and Cummins, who were influenced either directly or indirectly. There are many others whose contributions were less extensive but nevertheless important. It is not given to many men to be so productive both through their own efforts and through their influence on the efforts of others.

Dr. Arthur was a member of Sigma Xi; the American Society of Naturalists; the American Philosophical Society; the Academy of Natural Sciences of Philadelphia; the American Academy of Arts and Sciences; the Indiana Academy of Science (president, '93); the American Association for the Advancement of Science (vice president, '95); the Society for the Promotion of Agricultural Science; the Botanical Society of America (twice president, '02, '19); the Torrey Botanical Club; the American Phytopathological Society (president, '33); the Mycological Society of America; the Deutsche Botanische Gesellschaft; and the Russian Botanical Society.

The life and work of Dr. Arthur should serve as a great inspiration to ambitious young workers. A pioneer spirit, real resistance to discouragement, industrious habits, sound scholarship, unflagging persistence, and singleness of purpose led Dr. Arthur to high achievement.

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[1] Grateful acknowledgment is made to Dr. George B. Cummins who prepared the accompanying bibliography.

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A TOXIMETRIC STUDY OF SOME ERADICANT FUNGICIDES[1]

J. B. Carpenter

(Accepted for publication February 14, 1942)

INTRODUCTION

Chemical eradication is an accepted practice in the control of several major plant diseases and offers possibilities for wider use. In recent years renewed interest in this means of plant disease control has been stimulated by an increasing knowledge of epidemiology and by frequent failures of control programs that relied solely on applications of protectant fungicides. Special attention has been given to the feasibility of eradicant spraying as an aid in the control of certain diseases of orchard and small-fruit crops, particularly apple scab, caused by *Venturia inaequalis* (Cke.) Wint. (12, 16, 20). Several investigators (1, 2, 3, 7, 8, 10, 11, 13, 14, 15, 16, 17, 20, 21, 22, 23, and 28) have reported a drastic reduction of the ascosporic inoculum of this fungus by experimental applications of eradicant fungicides to the overwintered leaves on the orchard floor. Of the materials that have been thus tested, a proprietary preparation of sodium dinitro-ortho-cresylate, trademarked Elgetol, seems to have given the most satisfactory results.

The first comprehensive work on the toxicity to fungi of sodium dinitro-ortho-cresylate was reported in 1912 by Falck (5). Among the many cresol and phenol derivatives tested, the sodium and potassium salts of dinitro-ortho-cresol were the most toxic. Truffaut and Pastac (29), 1932, recommended the use of Elgetol, developed by them during a study of fungicidal organic dyes, as a fungicide and insecticide for dormant spraying. They cited numerous phytopathogens and insect pests against which it might be used. Pastac (25) has given further data on the toxicity of some dinitro compounds.

The purpose of the *in vitro* experiments reported here has been to study comparatively the toxicity of Elgetol and some other potential eradicant fungicides.

The test fungi were *Venturia inaequalis* and some other plant pathogens that might be combated by eradicant spraying.

The test materials, in addition to Elgetol, were Lignasan (a commercial preparation of ethyl mercury phosphate) and preparations of phenyl mercury oleate and a toluene derivative, respectively. Limited data of Keitt, Clayton, and Langford (16) have indicated that Lignasan has high toxicity to the scab fungus in overwintered leaves. The other 2 materials were chosen to include another organic mercurial compound and a toluene derivative that showed a promising degree of toxicity in preliminary tests.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

The writer expresses his appreciation to Dr. G. W. Keitt and Dr. B. M. Duggar for their supervision and advice during the course of this study.

Grateful acknowledgment is made to the persons who contributed fungi and to Mr. Eugene Herrling for preparing the text figures.

The procedure was based on the agar-plate method developed for toximetric studies of wood preservatives by Schmitz and others (27). Palmiter and Keitt (24) adapted the method to an *in vitro* study of eradicant fungicides. Humphrey and Fleming (9) have reviewed the methods and materials used in the early toximetric investigations of wood preservatives.

The method is adapted to a preliminary evaluation of the toxicity of potential fungicides and studies on their toxic action under the experimental conditions. Tests may be made without seasonal limitations, rapidly, and in a controlled environment. Critiques of the method, as applied to the testing of wood preservatives, have been presented by Bateman (4), Findlay (6), and Richards (26).

This work is designed to supplement field studies and aid in the interpretation of their results. It is recognized that the findings from such work cannot be applied directly to field practice because of the differences in conditions.

In addition to the *in vitro* studies, limited experiments were made on the suppression of the ascosporic inoculum of *Venturia inaequalis* in naturally overwintered apple leaves.

TOXIMETRIC STUDIES

Methods

The fungicides were pipetted aseptically from freshly prepared self-sterilized stock solutions or suspensions into proper volumes of cool sterile distilled water in 250-cc. Erlenmeyer flasks to give 100 cc. of fungicide at twice the desired final concentration. As certain of the fungicides had volatile components, none of them was heated at any time. The medium (Trommer's extract of malt, 25 g.; agar, 17 g.; and distilled water to make 1000 cc.) was freshly prepared at twice the final concentration, dispensed without filtering in 100-cc. lots into 250-cc. flasks, and autoclaved at 15 pounds' pressure for about 25 minutes. The medium was never reheated after the first sterilization.

The diluted fungicide was added to the partially cooled medium, shaken well, and about 50 cc. of the mixture poured into each of four 12-ounce bottles (Illinois-Owens ovals) with metal screw caps containing oiled-paper inserts. The bottles were laid on their broad plane surface while the medium gelled. For toxic materials in suspension, the temperature was regulated to provide rapid gelling. In adapting the method for the present study, Petri dishes were replaced by bottles to obviate appreciable losses of water and volatile components of the toxic agents.

The plates from which disks were cut for planting the bottles contained 15 to 17 cc. of malt-agar medium and were planted with a standardized water suspension of mycelium and spores. They were wrapped with wax paper and incubated in darkness at 70° F. for the following periods: *Sclerotinia fructicola* (Wint.) Rehm, 5 days; *Coryneum beijerinckii* Oud. and *Valsa cincta* Fr., 7 or 8 days; *Cladosporium carpophilum* Thüm, and

Coccomyces hiemalis Higgins, 14 or 15 days; and *Venturia inaequalis*, 16 or 17 days. Disks of *Valsa cincta* were cut before the pycnidia were visible or when only immature pycnidia were present.

Disks 5 mm. in diameter and of uniform thickness were cut from vigorous mycelia of the test fungi. Six disks with the fungus-bearing surface uppermost were placed about equidistant from each other on the medium in the bottle. The bottles were inverted, incubated in a horizontal position, and kept in darkness at 70° F. for 2 weeks. The liquid that accumulated on the lower inside surface of the bottle during the incubation period was carefully poured out before the disks were removed.

After the incubation period, the disks without macroscopic evidence of mycelial growth, subsequent to planting, were removed to Petri dishes containing about 20 cc. of malt agar and placed with the fungus-bearing surface next to the medium. The plates were inverted and incubated for 3 weeks. The fungus was considered dead if new growth did not appear. Each bottle was considered as a unit, being listed as positive if growth occurred on any disk. Care was taken to handle each disk separately when testing for viability, and all were recorded individually in the original data.

The criteria of toxicity are comparable to those used by Palmiter and Keitt (24): *growth* represents the highest concentration at which survival occurred in any series of tests, and *death* represents the lowest concentration at which killing always occurred in any series of tests.

Duplicate bottles for each concentration of fungicide and each isolate under consideration were prepared in every test. Every test to determine the critical data was done at least 3 times with freshly prepared fungicide and nutrient medium. Duplicate bottles containing plain malt-extract agar were planted for each isolate concerned in every experiment. The fungus always grew well in these bottles.

The test fungi grew well throughout the range of pH encountered in this work. Plain malt-extract agar had a reaction of pH 5.5 to pH 6.0. Every fungicide reduced the acidity of the medium, usually to the range of pH 6.0 to pH 6.5. At the higher concentrations of Elgetol the reaction of the medium was about pH 7.0. There were no appreciable pH changes during the test period, except when the fungus grew on the toxic substratum. Then the reaction shifted towards pH 5.5, the magnitude of the shift depending upon the extensiveness of the mycelial growth. All pH measurements were made with a Coleman Model–3 pH Electrometer.

It is recognized that minor errors in percentage concentration of the toxic substratum are inevitable in dealing with the methods and materials used here. All feasible precautions were taken to minimize such errors.

Materials

Description and Source of the Toxic Preparations

Elgetol: The Elgetol used was a liquid proprietary preparation containing 19.4 per cent by weight of sodium-dinitro-ortho-cresylate with water and a penetrant added.

Lignasan: The Lignasan used was a powdered proprietary fungicide containing 6.25 per cent by weight of ethyl mercury phosphate mixed with nontoxic inert material.

FD–2: Phenyl mercury oleate was used in a preparation coded FD–2 that contained 10 per cent by weight of the toxic agent. The remainder of the product was mineral oil and an emulsifier.

IN–3102 A8: A toluene derivative was used in a preparation coded IN–3102 A8 that contained 50 per cent by weight of the toxic agent.

At the concentrations used, Elgetol was completely in solution, Lignasan and the toluene derivative preparation were primarily suspensions, and the phenyl mercury oleate preparation was dispersed in an emulsion.

The data on the composition of each material listed above are based on the manufacturer's statement. Elgetol was furnished through the courtesy of Standard Agricultural Chemicals, Incorporated. Lignasan, the phenyl mercury oleate preparation, and the toluene derivative preparation, were provided through the courtesy of E. I. du Pont de Nemours and Company.

Source and Purity of the Test Fungi

The test fungi were obtained from the following sources: *Cladosporium carpophilum*, a monoconidial isolate from a peach twig furnished by Donald Cation, East Lansing, Michigan, 1939; *Coccomyces hiemalis*, 2 monoconidial isolates from Montmorency cherry leaves at Madison, Wisconsin, 1940; *Coryneum beijerinckii*, a monoconidial isolate from each of 2 cultures isolated from peach and furnished by E. E. Wilson, Davis, California, 1939; *Sclerotinia fructicola*, a monoconidial isolate from each of 2 plums collected at Gays Mills, Wisconsin, 1939; *Valsa cincta*, a monoconidial and a monoascosporic isolate from E. M. Hildebrand, Ithaca, New York, 1939; *Venturia inaequalis*, 2 monoascosporic isolates of opposite sexual compatibility groups from the same ascus, obtained by M. H. Langford at Madison, Wisconsin, 1937 (*cf.* 19). Isolate B4 is here designated as isolate *1*, and B8 as isolate *2*.

All of the isolates except those of *Valsa cincta* were propagated by monoconidial transfers, and all remained true to type, as judged by macroscopic characters. Usually 2 isolates of each organism were used to guard against the possibility of limiting the work to a single isolate of unusual reaction.

EXPERIMENTAL RESULTS AND DISCUSSION

Toxicity of the Fungicides to the Test Fungi

The data from the individual tests, based on the criteria of toxicity defined elsewhere, are summarized in table 1. Some of the critical data indicate the maximum tolerance of the isolate as expressed in 1 divergent test. This occurred especially with the non-mercurial preparations. As the lethal concentration of a fungicide was approached, the number of disks surviving usually diminished, until often the critical concentrations were determined by the survival of only 1 or 2 of the 12 disks in duplicate bottles.

Coccomyces hiemalis was constantly one of the more susceptible species,

usually followed in order by either *Venturia inaequalis* or *Valsa cincta*. *Cladosporium carpophilum, Coryneum beijerinckii,* and *Sclerotinia fructicola* were commonly the more resistant organisms.

Differential susceptibility to the toxic action of a given fungicide occurred among the species of test fungi. This was accentuated by the weaker toxic preparations. The reaction of each isolate was quite uniform in a series of tests, considering the relative toxicity of the fungicide used, and

TABLE 1.—*Toxicity of 4 potential eradicant fungicides to 6 phytopathogenic fungi*

Test fungus and isolate number		Criteria	Critical concentrations[a] for			
			Elgetol	Lignasan	Phenyl mercury oleate preparation	Toluene derivative preparation
			Per cent	*Per cent*	*Per cent*	*Per cent*
Cladosporium carpophilum	1	Growth	0.075	0.0025	0.010	.....
		Death	0.100	0.0050	0.025	> 0.200
Coccomyces hiemalis	1	Growth	0.010	0.0010	0.005	0.025
		Death	0.025	0.0025	0.010	0.050
	2	Growth	0.010	0.0010	0.020	0.010
		Death	0.025	0.0025	0.030	0.025
Coryneum beijerinckii	1	Growth	0.050	0.0025	0.040	0.100
		Death	0.075	0.0050	0.050	0.200
	2	Growth	0.100	0.0010	0.040	.....
		Death	0.200	0.0025	0.050	> 0.200
Sclerotinia fructicola	1	Growth	.....	0.0025	0.020	0.020
		Death	> 0.200	0.0050	0.030	0.030
	2	Growth	0.100	0.0050	0.030	0.030
		Death	0.200	0.0075	0.040	0.040
Valsa cincta	1	Growth	0.025	0.0025	0.005	.....
		Death	0.050	0.0050	0.010	> 0.200
	2	Growth	0.050	0.0010	0.020	.....
		Death	0.075	0.0025	0.030	> 0.200
Venturia inaequalis	1	Growth	0.050	0.0010	0.005	.....
		Death	0.075	0.0025	0.010	> 0.075
	2	Growth	0.050	0.0010	0.005	.....
		Death	0.075	0.0025	0.010	> 0.075

[a] The figure shown for growth is the highest concentration at which survival occurred in any series of tests; for death, the lowest that always killed.

> indicates that the lethal concentration was greater than the figure shown. Percentage concentrations are based on the toxic preparation as described in the text.

noteworthy differences between isolates of the same organism were not found. No correlation between the cultural characteristics of the several isolates and their susceptibility to toxic action was evident.

Under the experimental conditions, Lignasan was the most toxic fungicide, followed by the phenyl mercury oleate preparation, Elgetol, and the toluene derivative preparation. Based on equal percentage concentrations of the toxic agent, the toxicity of Lignasan was 3 to 300 times that of any other preparation, depending upon the fungicide and the isolate used. The phenyl mercury oleate preparation on this basis was considerably more toxic than Elgetol.

In considering the relative toxicity of these fungicides, allowance should be made for the advantages accruing to preparations yielding a toxic vapor. Except Elgetol, every preparation had a vapor that was toxic at the concentrations used, and that of Lignasan was especially toxic, as proved by limited experiments not reported in detail.

The specificity of the fungicides was brought out by the reactions of the several test fungi. Each mercurial preparation was consistently toxic within its own range to all of the isolates. Some evidence of specificity was found with Elgetol. The toluene derivative preparation was markedly toxic to only 2 test fungi, *Coccomyces hiemalis* and *Sclerotinia fructicola*, ordinarily a resistant species in this study and in that of Palmiter and Keitt (24).

Relation of Concentration of Toxic Preparation to Time Required for Killing Fungus

Indications were sought as to the time required for a given concentration of a fungicide to kill a particular fungus under the conditions used for studying relative toxicity. Elgetol and Lignasan were the fungicides used; the fungi were 2 isolates of each *Coryneum beijerinckii*, *Sclerotinia fructicola*, and *Venturia inaequalis*.

The methods of preparing the toxic substratum and the plates from which disks were cut were the same as those described earlier. Each bottle was planted with 6 disks and incubated for a suitable period under conditions given above. Thereafter, the disks of each bottle were removed to a Petri dish containing about 30 cc. of sterile distilled water, leached for approximately 1 hour, and then placed with the fungus-bearing surface down on malt-agar plates, which were inverted and incubated at 70° F. for 3 weeks. Disks that did not show signs of life after this interval were considered dead. Each bottle was considered as a unit, for the disks mingled during leaching.

The leaching extended the time during which the fungus was in contact with the fungicide, but this source of error is regarded as unimportant. The vitality of the fungus was not affected perceptibly by immersion, as shown by tests of untreated fungus-bearing disks.

The critical data were obtained by using each fungus with each concentration of the 2 fungicides and at each critical time interval in at least 3 tests, with 2 exceptions. Only 2 tests were made with *Sclerotinia fructicola* and Elgetol, at 0.25 per cent, after earlier data showed that Elgetol, at 0.10 per cent, was not lethal to either isolate in the 14-day test. One test was made with this fungus and Lignasan at 0.005 per cent. The results of the several tests in this part of the study were reasonably uniform.

Figures 1 and 2 indicate the maximum survival period for each isolate in any test of a given concentration of fungicide. Killing occurred at some time during the last recorded interval on the bar graph.

With both Elgetol and Lignasan, *Sclerotinia fructicola* was the most

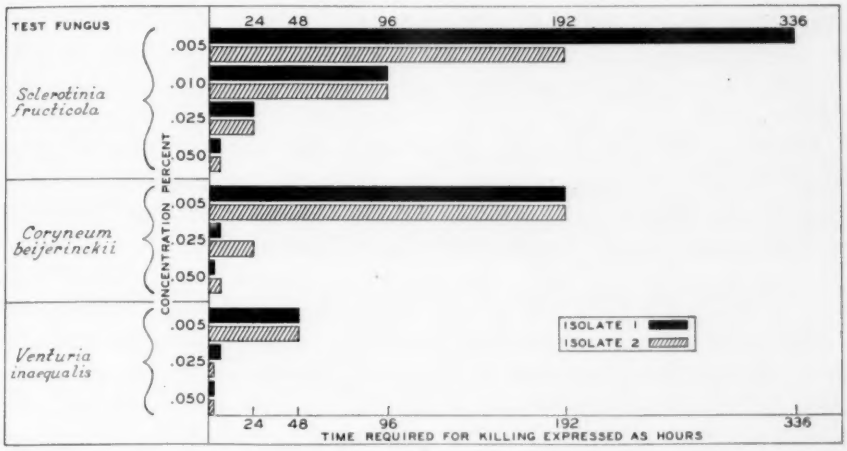

FIG. 1. Relation of the concentration of Lignasan to the time required for killing certain fungi.

resistant species, followed in order by *Coryneum beijerinckii* and *Venturia inaequalis*. Lignasan was the more toxic of the fungicides. The difference in susceptibility noticeable among the test fungi at the lowest concentration of Lignasan may be misleading unless it is observed, in table 1, that the concentration of 0.005 per cent was about a minimum lethal dose for *Sclerotinia fructicola*, 1 to 2 times that for *Coryneum beijerinckii*, and 2 to 5 times that for *Venturia inaequalis*. At a concentration of 0.025 per cent, the relations to the minimal lethal dose for these fungi were, respectively, 5, 5 to 10, and 10 to 25; at 0.05 per cent, 10, 10 to 20, and 20 to 50.

The fungi were killed rapidly at the higher concentrations of Lignasan. At 0.025 per cent, all of the isolates were killed within 24 hours. No more than 6 hours was required for killing at 0.05 per cent. Lignasan probably had important advantages over Elgetol in these tests because of its highly

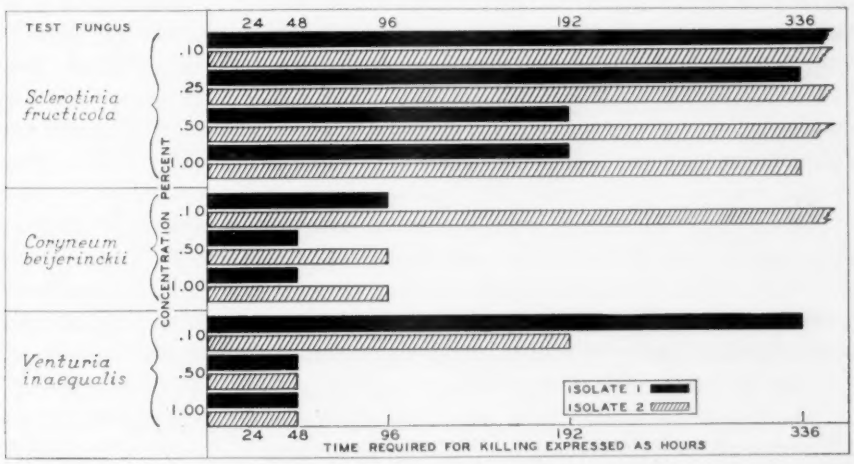

FIG. 2. Relation of the concentration of Elgetol to the time required for killing certain fungi.

toxic vapors and the use of an airtight testing chamber. Even at the highest concentration of Elgetol, *Sclerotinia fructicola* was markedly resistant, whereas the most resistant isolates of the other test fungi were killed within 48 to 96 hours.

Elgetol, at 0.5 per cent, was about twice the minimum lethal dose for *Sclerotinia fructicola,* 2 to 5 times that for *Coryneum beijerinckii* and 7 times that for *Venturia inaequalis.* At the concentration of 1.0 per cent, the relations were, respectively, about 5, 5 to 10, and 13.

Suppression of Ascosporic Inoculum of *Venturia inaequalis*

Data with which to supplement the *in vitro* experiments were sought in experiments at the field laboratory at Gays Mills, Wisconsin. Overwintered apple leaves bearing numerous perithecia of *Venturia inaequalis* were selected under trees of the Cortland variety on April 9, 20, and 25, respectively, for the 3 experiments reported here. Only leaves overwintered on sod with their dorsal side uppermost were used. Suitable numbers of leaves were arranged out-of-doors on sod under wire netting with the weathered surface exposed and no overlapping. They were sprayed with Elgetol, at 0.5 per cent, at the rate of 600 gallons per acre. A knapsack type Meyer's sprayer was used.

After the leaves were dry—within 2 hours after spraying—a single disk 15 mm. in diameter was cut from each in an area where ascocarps were abundant. At intervals of 3, 6, 12, and 24 hours after the leaves were sprayed, 20 disks for each time interval were leached in 1 liter of tap water for an hour. Then 5 disks were placed on the inside surface of the cover in each of 4 Petri dishes, the excess water was removed, and the perithecia were allowed to discharge ascospores for several hours on agar gel made aseptic by copper sulphate. Each disk was set up 4 consecutive times for ascospore discharge over the same area of the agar plate. Between discharges the disks were kept under conditions suitable for maturing of more ascospores. The discharges were made at 3- or 4-day intervals and at room temperature. Unsprayed checks of 20 disks each received the same treatment during the period of ascospore discharge. The abundant ascospore discharge from the perithecia in untreated leaf disks indicates that the experimental material and procedure were suitable for the purpose (Table 2).

The ascospores were counted under the microscope without duplication by manipulating the open Petri dish on a mechanical stage. When no more than a few hundred spores were present, all were counted or closely estimated. If several hundreds or thousands of spores were present, 30 fields per individual area were counted or closely estimated and the total discharge calculated.

The indicated reduction in ascospore discharge from perithecia in leaf disks treated with Elgetol varied little in the 3 experiments, usually amounting to more than 99 per cent (Table 2).

Evidently, Elgetol acts quickly, for the suppression after 3 hours was as complete as that at the longer intervals. The results of a single experiment suggest that rapid drying may hasten the inactivation of the fungus. In experiment 3, parallel series of 20 disks each were prepared for the 3-, 6-, and 12-hour intervals. The disks of 1 series were not allowed to dry during the interval from spraying to leaching, and the other series was handled in the usual manner. In leaves allowed to dry rapidly, the suppression was almost 100 per cent by the end of the 3-hour interval. Six hours were required for equal suppression of the fungus in the constantly wet leaves, although the suppression was about 98 per cent after the 3-hour treatment.

TABLE 2.—*Time required for suppression of the ascosporic inoculum of Venturia inaequalis by Elgetol applied to overwintered leaves of the Cortland variety, Gays Mills, Wisconsin, 1941*

Period of treatment in hours	Total ascospore discharge from 20 disks[a]			Indicated percentage reduction in discharge by treatment		
	Experiment No.			Experiment No.		
	1	2[b]	3	1	2	3
Untreated check	424,676	743,213	752,911			
3	92	19,818	5	99.9	97.9	99.9
6	204	4,090	534	99.9	99.6	99.9
12	707	21,715	144	99.8	97.7	99.9
24	149	12,192	1,340	99.9	98.7	99.8

[a] A single disk 15 mm. in diameter was cut from an area of a leaf where ascocarps were abundant.

[b] Most of the spores were from 1 or 2 disks: 3 hours, 2 disks with 11,084 and 5,444 spores, respectively; 6 hours, 1 disk with 2,406 spores; 12 hours, 1 disk with 18,635 spores; and 24 hours, 1 disk with 10,413 spores.

Interest attaches to the question whether Elgetol treatment can effectively suppress the ascosporic inoculum of the scab fungus if applied after the ascospores are ripe and ready for discharge. The developmental stages of the ascocarps and the course of natural discharge of ascospores at the time of the experiments were studied. Both fresh and preserved ascocarps were examined in the study of developmental stages. The preserved material was chosen from treated leaves just before the disks were set up for the first ascospore discharge in each experiment and was fixed in formal-acetic alcohol, embedded in paraffin, sectioned, and stained in 0.05 per cent aqueous cotton blue. Perithecia were also picked from the preserved leaves, crushed, and examined.

Ascospores were starting to mature at the beginning of the first experiment, so that a few olivaceous spores were present in some of the perithecia. At the beginning of the second and third experiments, many of the ascocarps contained not less than 5 to 10 asci with olivaceous spores (Fig. 3).

Evidence on natural discharge of ascospores was obtained by the method of Keitt and Jones (18). The first natural discharge was observed on April

9, and small to moderate discharges occurred by the time of the second and third experiments.

It is noteworthy that the suppression of the ascosporic inoculum in the second and third experiments was as effective as in the first.

The *in vitro* experiments reported elsewhere in this paper necessitated the use of killing as the criterion of effectiveness of the several fungicides. In practice, the goal of chemical eradication is the suppression of the inoculum of a phytopathogen. Consequently, immediate killing may be of secondary importance. It is significant that Elgetol, at 0.5 per cent, drastically suppressed the inoculum of *Venturia inaequalis* in overwintered apple leaves within 3 hours, whereas, in *in vitro* experiments, 24 to 48 hours elapsed before all of the fungus was killed at this concentration.

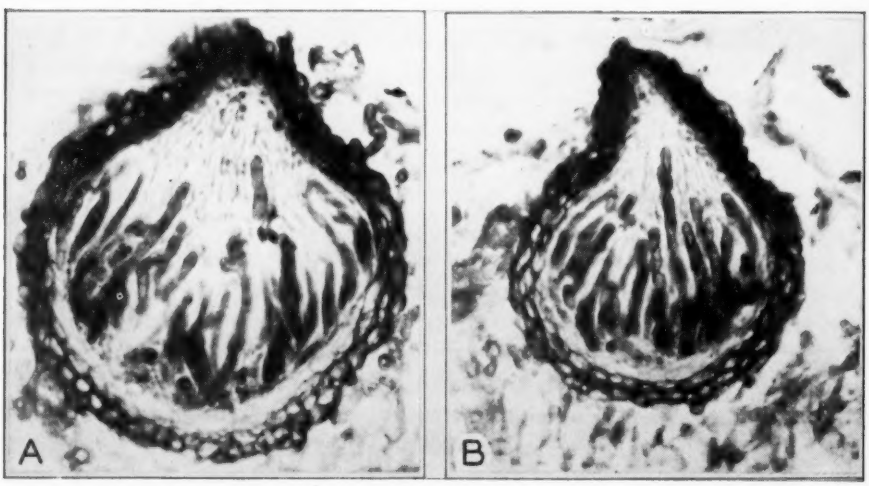

FIG. 3. Sections of perithecia of *Venturia inaequalis* from material fixed at the beginning of the second experiment, illustrating the more advanced developmental stages observed. A. Untreated perithecium. B. Perithecium treated with 0.5 per cent Elgetol. Both approximately × 330.

A satisfactory suppression of the inoculum of certain fungi is most sure with toxic preparations that act quickly. For instance, the apple-scab pathogen and *Coccomyces hiemalis,* the cherry leaf-spot pathogen, develop on the orchard floor in overwintered leaves from which spray residue may be leached by rain. A preparation that at feasible concentrations would render a sufficient part of the potential inoculum harmless within a few hours is the type of material sought. There are strong indications from the orchard trials of other investigators that Elgetol and Lignasan approach this requirement. The results of the field experiments reported herein give additional evidence that Elgetol is capable of rapid and drastic suppression of the ascosporic inoculum of *Venturia inaequalis.*

SUMMARY

The toxicity of 4 potential eradicant fungicides was studied *in vitro* by a modification of the agar-plate method of Schmitz and others, 6 species of

phytopathogens being used as test fungi. Limited studies were made of the suppression of the ascosporic inoculum of *Venturia inaequalis* in overwintered apple leaves by Elgetol.

Coccomyces hiemalis was constantly one of the more susceptible test fungi, followed in order by either *Venturia inaequalis* or *Valsa cincta*. *Cladosporium corpophilum, Coryneum beijerinckii,* and *Sclerotinia fructicola* were more resistant. The relative susceptibility of the organisms varied with each toxic preparation. Each isolate reacted quite uniformly in a series of tests, and the differences between isolates of the same species were not noteworthy.

Under the experimental conditions, Lignasan was the most toxic fungicide, followed in order by the phenyl mercury oleate preparation, Elgetol, and the toluene derivative preparation. These materials usually killed at relatively low concentrations. Each, except Elgetol, had a toxic vapor at the concentration used, an advantage that should be considered in interpreting relative toxicities. Specificity of toxic action was shown clearly only by the toluene derivative preparation.

Venturia inaequalis was killed within 3 hours by Lignasan and within 24 to 48 hours by Elgetol at the highest concentrations used in the *in vitro* experiments. *Coryneum beijerinckii* was more resistant, and *Sclerotinia fructicola* was the most resistant organism to both fungicides. Although Elgetol killed more slowly than Lignasan, the apparent difference in rate of killing becomes less important if the relation between the minimum lethal dose and the concentration used for each fungus and fungicide is considered.

Elgetol at 0.5 per cent, used at the rate of 600 gallons per acre, usually suppressed more than 99 per cent of the ascosporic inoculum of *Venturia inaequalis* in naturally overwintered apple leaves. Suppression was as complete 3 hours after treatment as later. It was possible in these experiments to limit drastically the ascospore discharge from perithecia containing ripe spores.

The importance of the time required for adequate suppression of the ascosporic inoculum of certain fungi is discussed.

The results reported herein give additional evidence that Elgetol is capable of rapid and drastic suppression of the ascosporic inoculum of *Venturia inaequalis*.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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THE EFFECT OF MOSAIC VIRUS INFECTION ON THE PROTEIN CONTENT OF SUSCEPTIBLE AND RESISTANT STRAINS OF TOBACCO[1]

CLAUDE H. HILLS AND H. H. MCKINNEY[2]

(Accepted for publication February 23, 1942)

INTRODUCTION

It is known that the common-mosaic virus causes an alteration in the normal protein synthesis and that the multiplication of virus in susceptible strains of tobacco is influenced by the nitrogen nutrition of the plants.

Stanley (17) found that the extracts from the mosaic-diseased plants contained more total nitrogen and more protein than the extracts from healthy plants of the same age. Bawden and Pirie (4) also observed the abnormally high protein content of the expressed juice from mosaic-diseased tobacco plants.

Spencer (15) found that Turkish tobacco plants grown on a very low nitrogen level produced little, if any, virus, whereas an increased nitrogen supply accelerated the synthesis of virus protein.

The changes in various protein fractions of mosaic-diseased tobacco were studied by Martin, Balls, and McKinney (10, 11). However, since all the protein of the plant is not in the expressed juice, they made use of the tissues, as well as the juice, in their determinations. They concluded that during the early stages of infection the virus protein accumulates by displacing an equal amount of normal protein. At a later stage the virus protein is present in addition to the normal amount of soluble proteins maintained by healthy plants. They made the interesting observation that some strains of tobacco that are extremely resistant to the mosaic virus show a decrease in total nitrogen content as a result of virus infection (11). This response is entirely opposite to that observed in the susceptible varieties of tobacco investigated.

In the present study we have investigated further the differences in the protein metabolism and the oxidizing enzyme contents of susceptible and resistant types of tobacco. Such studies are of value in arriving at an understanding of the mode of virus synthesis in plants and the basis of virus resistance exhibited by various genotypes of tobacco.

EXPERIMENTAL

Materials and Methods

The tobacco plants used in this study were of two types: (1) *Nicotiana tabacum* L., var. Wisconsin-Havana Seed—a mosaic-susceptible variety—

[1] Studies conducted under Bankhead-Jones Project S.R.F. 2–17, U. S. Department of Agriculture, Bureau of Plant Industry and Bureau of Agricultural Chemistry and Engineering cooperating.

[2] Associate Chemist, Bureau of Agricultural Chemistry and Engineering; senior pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., respectively.

and (2) T.I. No. 448A, a genotype of common tobacco, which was found by McKinney (8) to possess the highest degree of resistance to the tobacco-mosaic virus known in tobacco. Although T.I. No. 448A develops a low concentration of virus in the leaves, it does not develop the usual mosaic symptoms, and the virus is not completely systemic in the plant.

The plants were grown in the greenhouse in pots during the winter and spring. Temperatures were controlled near a mean of 22.5° C., but this temperature was exceeded on days when the sun was bright. Plants were inoculated with *Nicotiana Virus 1* when the largest leaves were from 3.5 to 5 cm. wide. In the low-nitrogen series the plants were potted with a very poor soil, low in humus and soluble nitrogen. The series designated as "good soil" was grown in composted greenhouse soil made up from fertile field soil and composted manure. In the high-nitrogen series, the plants were potted in "good soil" and to each pot was added one pint of a 1.0 per cent solution of calcium nitrate twice a week.

Plant collections were made when the symptoms became fully developed in Wisconsin-Havana Seed tobacco or, in some instances, at an arbitrary time after inoculation. Four or more plants from each set were cut at the point of inoculation and all the leaves above the inoculated leaf, including the tip leaves, were removed and the stems discarded. The leaves were then cut into small pieces with a knife and mixed to give uniform samples for the determination of dry matter and total nitrogen. Total nitrogen was determined on the oven-dry samples by the usual Kjeldahl method, except that the sulphuric acid used for the Kjeldahl digestion contained 3 g. salicylic acid per 100 cc. of acid.

The remaining fine-cut leaf tissue was frozen and later thawed and extracted with an equal weight of M/10 phosphate buffer, pH 7.0. After standing at room temperature for 2 or 3 hours the tobacco-leaf extract was squeezed through cheesecloth and clarified by centrifuging. Aliquots of this extract were used for the determination of soluble-protein nitrogen, non-protein nitrogen, virus nitrogen, and virus assays on bean leaves by the paired-leaf method.

The nonprotein nitrogen was measured by the iron-reduction method of Pucher, Leavenworth, and Vickery (13). Protein nitrogen was determined by precipitation with 2.5 per cent trichloroacetic acid and heating at 75° C. for 10 minutes. The precipitate was resuspended in a 10-cc. centrifuge tube with 2.5 per cent trichloroacetic acid and then centrifuged. The washed precipitate was dissolved by gently heating with 1 cc. of concentrated sulphuric acid and then transferred to a micro-Kjeldahl digestion flask. The nitrogen was determined by the micro-Kjeldahl method.

Virus-protein nitrogen was determined by the isoelectric precipitation method described by Hills and McKinney (7). In brief, the procedure was to acidify a 40-cc. aliquot of the leaf extract to pH 4.2–4.0, at which point a fraction of nonvirus protein precipitates and can be removed by centrifuging. On careful acidification to pH 3.4 the virus nucleoprotein is ob-

tained in the precipitate and the virus-protein nitrogen can then be determined in the same manner as protein nitrogen.

Biological assays of the virus were made by the paired-leaf method on the primary leaves of 8- to 12-day-old bean seedlings (*Phaseolus vulgaris* L., var. Scotia).

The determinations of chlorophyll and chlorophyllase in fresh green leaf tissue were made by the method used by Peterson and McKinney (12). The midribs and petioles were removed in order to get more uniform and reliable leaf samples.

The extracts used for the estimation of oxidase, catalase, and peroxidase were prepared by grinding 10 g. of fresh, fine-chopped leaf tissue in a mortar with sand and 100 cc. of a mixture of 1 part glycerol and 1 part of M/5 phosphate buffer, pH 8.0. The suspension was centrifuged at a very slow speed (about 700 r.p.m.) for 5 minutes to remove coarse fiber and sand. The solution was stored in a refrigerator at 0° C. Determinations were made within 3 days after preparation of the glycerol extracts. It was found that the enzymes being studied did not deteriorate measurably in 3 days' storage at 0° C.

Oxidase activity was measured by Guthrie's (6) iodometric method. Catalase and peroxidase were determined by the methods published by Balls and Hale (2, 3), except that it was found convenient to remove aliquots immediately and after 4, 8, and 12 minutes instead of after the time intervals suggested by those authors. Catalase and peroxidase activities are expressed as the reaction velocity constant "K" for 1 g. of fresh leaf material.

RESULTS

Table 1 shows that Wisconsin-Havana Seed tobacco responded to virus infection with an increase in total nitrogen content in the diseased leaves. However, the virus concentration in extracts of the nitrogen-deficient plants was nearly normal. The plants grown on poor soil were less than half the size of those grown on an average greenhouse soil. Spencer's (16) observation that tobacco plants grown on a nearly nitrogen-free sand contained only 1/30 the virus content of plants grown in soil does not necessarily conflict with our results. His nitrogen-deficient plants received less than 10 per cent of the amount of soluble nitrogen fed to the medium-nitrogen series, and made only 1/9 the normal amount of growth. Under such conditions of severe nitrogen starvation he was able actually to reduce the virus concentration after the first systemic spread.

The response of T.I. 448A tobacco grown on good soil was erratic. The average of 6 experiments shows a slight increase in total nitrogen of the diseased plants as compared to the healthy controls. The decreased nitrogen content of the diseased plants grown on poor soil indicates that the effect of virus infection on the resistant T.I. 448A tobacco is influenced by the level of nitrogen supply. Assays on T.I. 448A plants showed very low levels of virus concentration, even at the stage of maximum virus concentration, and in some instances the amount was too small to show up in a 10^{-1} dilution.

TABLE 1.—*The influence of common-mosaic virus on the total nitrogen content of susceptible and resistant tobacco plants cultured at different levels of nitrogen supply. Percentage of nitrogen based on dry weight of tissue*

No. of experiment	Days from inoc. to analysis	Poor soil			Good soil			Good soil plus nitrogen		
		Healthy plants	Diseased plants		Healthy plants	Diseased plants		Healthy plants	Diseased plants	
		Nitrogen	Nitrogen	Lesions per test leaf	Nitrogen	Nitrogen	Lesions per test leaf	Nitrogen	Nitrogen	Lesions per test leaf
	No.	*Per cent*	*Per cent*	*No.*	*Per cent*	*Per cent*	*No.*	*Per cent*	*Per cent*	*No.*
Wisconsin-Havana Seed Tobacco (Susceptible)										
I		.....	.....	.....	3.70	4.35	28.9[a]	5.75	6.39	25.7[a]
II	16	.....	.....	.....	4.57	5.35	15.1	5.81	6.57	9.6
II	26	.....	.....	.....	3.39	4.31	35.9	4.74	5.38	43.1
IV	23	.....	.....	.....	3.96	5.08	32.7	.....	.....	.....
V	24	3.74	4.35	19.4[a]	3.24	4.50	25.1	5.92	6.56	25.5
T.I. 448A Tobacco (Resistant)										
II	16	.....	.....	.....	5.15	4.98	.32[a]	4.74	5.38	.46[a]
II	26	.....	.....	.....	3.60	3.37	4.3	4.48	5.00	29.7
III	9	3.43	3.07	3.8[a]	4.81	4.87	7.3	.....	.....	.....
III	21	3.62	2.61	0.0	3.16	3.79	0.0	.....	.....	.....
III	32	2.98	2.98	0.0	2.78	2.79	0.0	.....	.....	.....
IV	23	.....	.....	.....	5.13	5.55	48.8	.....	.....	.....

[a] Virus extracts from Wisconsin-Havana Seed tobacco were diluted to 10^{-3} and those from T.I. 448A tobacco were diluted to 10^{-1} in phosphate buffer at pH 7.0. Each sample was then wiped on 30 bean leaves.

TABLE 2.—*The influence of common-mosaic virus on the nitrogen fractions and virus-protein in susceptible and resistant tobacco cultured at two levels of nitrogen supply. Percentages of nitrogen based on dry weight of tissue. Analyses made 24 days after inoculation*

Plants	Good soil					Good soil plus nitrogen				
	A	B	C	D	E	A	B	C	D	E
	Nitrogen (total)	Soluble protein nitrogen	Non-protein nitrogen	Virus protein nitrogen	Lesions per test leaf	Nitrogen (total)	Soluble protein nitrogen	Non-protein nitrogen	Virus protein nitrogen	Lesions per test leaf
	Per cent	*Per cent*	*Per cent*	*Per cent*	*No.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*No.*
Wisconsin-Havana Seed Tobacco (Susceptible)										
Healthy	3.24	.109	.261	.....	.....	5.92	.172	1.36	.....	.....
Diseased	4.50	.168	.320	.091	20.1[a]	6.56	.264	1.33	.107	33.7[a]
T.I. 448A Tobacco (Resistant)										
Healthy	3.60	.099	.565	.....	.....	4.48	.095	.937	.....	.....
Diseased	3.37	.092	.498	[b]	4.3[a]	5.00	.106	1.275	[b]	29.7[a]

[a] Fresh-leaf samples from infected Wisconsin-Havana Seed tobacco were diluted to 10^{-3} and those from T.I. 448A tobacco were diluted to 10^{-1} in phosphate buffer at pH 7.0. Dilutions based on grams fresh weight per cc. of buffer. Tissues finely pulped in mortar, then brought to final dilution. Each sample wiped on 30 bean leaves.

[b] Virus protein concentration too low to measure by chemical methods.

A more detailed analysis of the effect of virus infection on the nitrogen metabolism of susceptible and resistant strains of tobacco is provided by the data in table 2. All analyses were calculated on the dry-weight basis. However, the dilutions used in making virus assays were calculated on the fresh-weight basis.

The virus concentration in extracts of T.I. 448A tobacco leaves was only 1 per cent of the virus concentration in extracts of Wisconsin-Havana Seed tobacco as determined by biological assay on bean leaves. The virus-protein content in T.I. 448A tobacco was too low to be determined accurately by the chemical method. Virus-protein nitrogen, as determined by the isoelectric precipitation method, is recorded in table 2, column D.

Wisconsin-Havana Seed tobacco plants infected with virus developed a relatively high concentration of the abnormal virus nucleoprotein. Although analysis of these plants showed an increase in the soluble-protein fraction, the increase did not equal the amount of abnormal virus protein included in the soluble-protein fraction. This means that the plant produced a reduced amount of normal protein in the presence of the virus protein. The plants grown on an increased nitrogen supply showed the same, though a less marked, replacement of part of the normal protein by virus protein. The changes in nonprotein nitrogen were more pronounced in the plants grown on an average nitrogen level than in those grown on a high nitrogen level.

The resistant strain T.I. 448A showed a much less marked response to virus infection than did the susceptible variety. Our results confirm those previously reported by Martin, Balls, and McKinney (11), who found that virus infection caused a decrease in the total-nitrogen content of a mosaic-resistant tobacco. The soluble-protein nitrogen and nonprotein nitrogen of T.I. 448A tobacco seem to parallel the total-nitrogen content of the plant and are not noticeably altered by the presence of mosaic virus. The virus content of this resistant strain never reaches a high level, and the plants do not develop mosaic symptoms.

A study was made of the effect of virus infection on the chlorophyll content and on the activity of some of the enzymes of susceptible and resistant tobacco plants. The plants were collected 23 days after inoculation, when the mosaic symptoms in Wisconsin-Havana Seed tobacco were fully developed. The samples included all of the mosaic-mottled leaves. Comparable tissues were collected from the healthy control plants. All determinations were made on freshly ground leaf tissue that had been freed of petioles and midribs. Owing to conditions not under the control of the authors, it was not possible to divide the samples on the basis of the several stages of tissue development. The data are arranged in table 3.

The decrease in chlorophyll and chlorophyllase observed in the diseased Wisconsin-Havana Seed tobacco agrees with the results of Peterson and McKinney (12).

The resistant strain, T.I. 448A tobacco, showed no changes in the concentration of leaf chlorophyll nor in the activity of the leaf chlorophyllase.

Oxidase and catalase were decreased by the virus in the susceptible and the resistant plants. However, in the case of peroxidase, there was a decrease in the susceptible plants and an increase in the resistant plants. In general, the changes in the enzyme contents were less in the infected resistant plants than they were in the infected susceptible plants.

Guthrie (6), using the iodometric method, found in mosaic-diseased tobacco 3 times as much oxidase as in healthy tobacco. Dunlap (5) made a point of separating the young from the mature tissues in his samples of tobacco tissue, with the result that he found a higher than normal respiration rate in the mature diseased tissue. Guthrie (6) may have used young leaves

TABLE 3.—*The influence of common-mosaic virus on the chlorophyll and the enzyme content of susceptible and resistant tobacco plants. Determinations made 23 days after inoculation*

Plants	Chlorophyll	Chlorophyllase	Oxidase	Peroxidase	Catalase
	Wisconsin-Havana Seed Tobacco (Susceptible)				
	Mm.	*Mm.*	*Cc.*	*"K"/g.*	*"K"/g.*
Healthy	20.0[a]	20.00[a]	3.580[b]	0.845[c]	1.600[c]
Diseased	29.0	27.60	2.400	0.630	0.765
	T.I. 448A Tobacco (Resistant)				
Healthy	22.5	18.00	3.000	0.890	1.345
Diseased	22.4	18.00	2.250	1.080	1.145

[a] Depth of solutions of equal concentration required to match standard in colorimeter.
[b] Oxidase activity expressed in cc. of N/100 solution of sodium thiosulphate per g. of fresh material with a reaction time of 1 hour at 30° C.
[c] Peroxidase and catalase activities expressed as the reaction velocity constant "K" per g. of fresh material.

or young plants in his studies, but this point is not clear. If the writers had been in a position to study tissues in different stages of development, it is quite possible that their results would have harmonized with those reported by Guthrie and by Dunlap.

DISCUSSION

In Wisconsin-Havana Seed tobacco, virus multiplied rapidly during the first 10 days or 2 weeks following inoculation (10, 11), and when the mosaic symptoms were fully developed the virus-protein concentration of the leaves amounted to 50 per cent or more of the soluble-protein nitrogen and approximately 2 per cent of the total nitrogen of the diseased leaves.

The increase in total nitrogen of susceptible tobacco plants infected with common-mosaic virus was confirmed by our present study. Wisconsin-Havana Seed tobacco infected with common mosaic showed an increase in total-nitrogen content, which was much greater than the actual increase in virus protein. This would seem to indicate that mosaic infection stimulated

the plant to synthesize an abnormally high amount of protein, the major portion of which was water-insoluble tissue protein, only a small fraction consisting of the virus protein itself.

Wisconsin-Havana Seed tobacco, grown on a high nitrogen level, was able to synthesize nearly the normal amount of water-soluble protein in addition to the large quantity of abnormal virus protein present, but plants grown on a lower nitrogen level showed a greater replacement of soluble protein by virus protein.

The mosaic-resistant strain (T.I. 448A) showed a much different response to virus infection. A decrease in total nitrogen was observed in plants grown with a low nitrogen supply. The virus concentration in such plants was very low and the leaves formed after inoculation contained little or no virus. Mosaic-resistant plants grown on the higher levels of nitrogen supply showed no decrease in total nitrogen, and in some instances an actual increase was observed. The virus reached a higher concentration, and the leaves formed subsequent to inoculation escaped infection at a later stage in the growth of the plants. It is apparent that the resistance of such plants to mosaic virus is modified to a limited extent by the level of nitrogen nutrition.

The effect of mosaic virus on the total nitrogen content of susceptible and resistant strains of tobacco has a commercial significance because with the more important types of tobacco a low nitrogen content is very desirable.

Evidence that viruses produce fundamental changes in the carbohydrates and enzymes of plants has accumulated from various sources (1, 5, 12). Dunlap (5) observed that, although mosaic virus caused an increase in the total nitrogen of tobacco, there was a marked decrease in carbohydrates. Respiration was higher in young tobacco plants infected with mosaic, but in older plants the respiration was decreased. The effect of age on the activities of various enzyme systems in mosaic-diseased tobacco may explain the increased oxidase activity observed by Guthrie (6) in mosaic-diseased tobacco leaves. Our results, derived from the total mosaic-diseased tissue from plants 23 days after inoculation, showed an actual decrease in oxidase activity as compared with healthy controls. We also observed decreases in the catalase, peroxidase, and chlorophyllase in the infected susceptible tobacco. The infected resistant tobacco showed a decrease in oxidase and catalase nearly equivalent to that observed in the infected susceptible variety, whereas the virus concentration in the resistant plants was only about 1 per cent that of the susceptible plants. This low concentration of virus was sufficient to produce a measurable disturbance in the metabolism of the plant, but it was not enough to induce visible signs of disorder.

An outline of the interrelationship of viruses and environmental factors in the metabolism of plants has been discussed recently by Selman (14). He suggests that the primary effect of plant virus infection on the metabolism of the plant is an abnormal protein synthesis, and that the presence of the virus protein affects the permeability of the tissues, transpiration, and the carbohydrate metabolism.

The exact sequence of the changes induced as a result of virus infection cannot be determined from existing data, but from the writers' (9) studies on the perennial pepper plant (*Capsicum frutescens* L.) it is reasonable to believe that the virus soon initiates reactions which result in one or more products that are destructive to many kinds of plants. In the case of the perennial pepper inoculated with *Nicotiana Virus 1* and cultured near 32° C., these products appear to move some distance from the sites of detectable virus, destroying the chlorophyll in mature leaves and occasionally inducing mosaic mottling in the young leaves.

Mosaic mottling in tobacco may be caused directly by the products of a deranged metabolism that is induced directly by the virus. However, in mosaic-susceptible tobacco, such as Wisconsin-Havana Seed, active virus is very closely associated with the chlorotic areas.

SUMMARY

A study was made of the effect of common-mosaic virus on the nitrogen content of a mosaic-susceptible tobacco (Wisconsin-Havana Seed) and a mosaic-resistant tobacco (T.I. 448A).

Virus infection caused a marked increase in the total-nitrogen content of susceptible tobacco grown under conditions of low, medium, and high nitrogen nutrition. The resistant tobacco, when grown with a reduced nitrogen supply, showed a decrease in total nitrogen in the virus-infected plants, but when the plants were grown with a more adequate nitrogen supply, they showed no decrease in total-nitrogen content.

Mosaic-diseased leaves of Wisconsin-Havana Seed tobacco contained 30 per cent less chlorophyll and had a lower chlorophyllase activity than healthy leaves. There was no change in the chlorophyll or chlorophyllase of virus-infected leaves of tobacco T.I. 448A.

The oxidase activity of both the resistant and susceptible strains of tobacco was decreased by mosaic virus, and, while the changes in the enzyme activities caused by virus infection were slightly less in the resistant than in the susceptible variety, it is evident that a very small amount of virus nucleoprotein was sufficient to produce measurable alterations in the metabolism of the mosaic-resistant tobacco.

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THE DOWNY MILDEW DISEASE OF OATS, CAUSED BY SCLEROSPORA MACROSPORA[1, 2]

L. E. Miles[3] and James M. Epps

(Accepted for publication February 2, 1942)

INTRODUCTION

The genus *Sclerospora* has attracted increasing attention during recent years because of the destructive parasitism of its species on a number of important gramineous host plants. Although several species of the genus are know to attack members of the grass family, only two have been found in the United States. These are *Sclerospora graminicola* (Sacc.) Schroet. and *S. macrospora* Sacc. Both are worldwide in their distribution and both occur in temperate and tropical regions. Our present knowledge of these fungi, particularly the latter, is scanty, and additional information relative to the appearance of either of these fungi in new localities or on additional host plants is of considerable importance to agriculture. Accordingly, when *S. macrospora* was found for the first time in Mississippi and for the first time on oats in the United States, it seemed necessary to investigate its potential destructive activity and to appraise the possibility of it becoming a menace to the oat crop, now rapidly attaining a position of importance in the Delta area along the Mississippi River, where this infestation was found.

The purpose of this paper is, therefore, to describe the symptoms of oat downy mildew, compare the causal organism with other species of the genus *Sclerospora*, and report results of preliminary studies on certain phases of its life cycle.

THE DISEASE

Distribution in Mississippi

The disease was discovered in the spring of 1939 by the senior writer (12) on oat specimens collected by a grower and submitted for diagnosis. They were collected in the vicinity of Indianola, Sunflower County, Mississippi, in the Yazoo-Mississippi Delta. The plants were in an advance stage of development, and, a large part of the crop having already been harvested, no extensive survey for determining the extent of the trouble was possible for that year. A limited search, however, showed the mildew present in numerous fields in the immediate neighborhood of Indianola and Inverness. The infested fields were sown to locally grown seed, which indicated that the disease probably had been present for some years, or at least that it was not of recent introduction. A brief survey in the spring of 1940 revealed its

[1] Published with the approval of the Director of the Mississippi Agricultural Experiment Station. Paper No. 49. New Series.

[2] The writers wish to express their gratitude to Dr. W. H. Weston for his kind assistance, special laboratory facilities, and encouragement during the course of these investigations; special thanks are also extended to Dr. J. A. Pinckard for assistance with the manuscript.

[3] Deceased, May 10, 1941.

presence in the same fields in which it had occurred in 1939. In a few instances the fields had been resown to the same crop. The disease also was found to be of common occurrence in the adjacent counties of Washington, Sharkey, and Leflore. It was later found in oat fields at the Mississippi Agricultural Experiment Station, State College, indicating that the disease was not confined to the Delta region where it was first observed. The disease seemed to be most severe in low fields that, at some time during the growing season, had passed through a period of flooding. In such areas it was not uncommon to find nearly all plants showing infection. In other fields, not subjected to flooding, affected plants were found distributed over the entire area, although not in epiphytotic proportions.

Sclerospora macrospora was found in the spring of 1940 parasitizing a few volunteer wheat plants growing in an oat field near Leland. Although a number of wheat fields were carefully examined, this was the only instance in which the fungus was found in Mississippi on any host other than oats. It is possible, however, that a more extensive survey would reveal its presence on other grain crops and on a number of wild grasses, such for example, as *Bromus commutatus* Schrad. (cheat grass), commonly occurring in oat fields, and according to Weston (29), prevalent and generally infected in the vicinity of Tennessee and Kentucky wheatfields.

Distribution in Other Countries

Although the report (12) of the disease on oats in Mississippi constituted the first record of the occurrence of *Sclerospora macrospora* on that host in the United States, it had previously been recorded on oats from New South Wales (13, 14, 15), Italy (16, 17, 18, 19) and France (1). The fungus was reported in the United States in 1921 by Weston (29) on wheat and cheat grass in Kentucky and Tennessee, and was reported the same year by Mackie (10) on wheat in California.

Sclerospora macrospora was first described by Saccardo (24) in 1890 on foxtail grass (*Alopecurus* sp.) from Caronby, Australia. Noble (14) states that photographic records indicate that the disease was present on wheat in Queanbeyan, Australia, as early as 1891. Traverso (27), in 1924, reported that spikes of grain from the Cryptogamic Laboratory in Pavia showed that the disease had been present in Italy since 1873. The disease has been reported on wheat and oats from the United States (12, 29), Italy (16, 17, 18, 19, 22, 25, 26, 28), France (1, 2), and Australia (6); on rice from Italy (4, 5, 8), Japan (30), and Australia (15); on corn from Italy (7, 9), and Australia (14); on barley from the United States (10), and Italy (25, 28); and on rye from Australia (14, 15) and Bulgaria (3). In addition, it has been collected on various wild grasses from all the above-mentioned countries and from Germany (11).

Following is a list of host plants on which the fungus, *Sclerospora macrospora*, is known to occur: *Agropyron repens* (L.) Beauv., *Agrostis alba* L., *Alopecurus* sp. L., *A. agrestis* L., *Avena fatua* L., *A. sativa* L., *Bromus com-*

mutatis Schrad., *Cyperus articulatus* L., *Eragrostis major* Host, *Festuca elatior* L., *Glyceria maritima* Wahlb., *Holcus mollis* L., *Hordeum sativum* Pers., *Lollium perenne* L., *L. temulentum* L., *Oryza sativa* L., *Panicum antidotale*, *Phalaris arundinacea* L., *P. canariensis* L., *P. coerulescens*, *Phragmitis communis* Trin., *Secale cereale* L., *Syntherisma sanguinale* Dulac, *Triticum sativum* L., and *Zea mays* L.

Symptoms on Oats

Many of the affected plants had a tendency to be stiff and were characterized by an upright leaf habit. Such plants showed no internodal shortening; the upright deformed heads frequently stood above the more drooping,

FIG. 1. Oat heads deformed by *Sclerospora macrospora*.

heavier panicles of unaffected plants. In some cases the uppermost leaves were curled and twisted about the poorly developed heads so that only small portions of the latter were visible (Fig. 1, A). Some plants produced a rachis of normal length, although so curled and twisted that it appeared much shorter than usual. A rachis of this type usually produced a small cluster of sterile spikelets at its tip (Fig. 1, B). In other cases the rachis was very much shortened and reduced to a small compact cluster of frayed and tangled spikelets (Fig. 1, C). Some plants exhibited a very peculiar deformation of the fruiting panicle. The rachis was variously coiled or much elongated and produced only a few sterile structures resembling spikelets. Instead of each spikelet bearing 2 seeds in close contact within the same glumes, there was a single seed-like structure that enveloped the rachilla. The latter, greatly enlarged and elongated beyond the tips of the

glumes, formed a node from which derived a second seed-like structure, very similar to the first, but lacking the enclosing glumes; the lemmas constituted the outer covering (Fig. 1, D). In some cases the rachilla, continuing to elongate, formed additional nodes sometimes bearing leaf-like structures. In other cases additional sterile seed-like bodies evolved. The rachilla frequently was of normal length and diameter, but became twisted about the rachis, giving a compact, elongated, head-like appearance to the fruiting panicle (Fig. 1, E).

In certain specimens of the type characterized by Fig. 1, D, the rachis was elongated as much as 4 or 5 inches between the sterile spikelets, or the sterile seed-like structures replacing them. The resulting panicle exhibited a scattered drooping aspect. Frequently the sterile seed-like structures were markedly elongate and somewhat curved. Occasionally, a single panicle showed all of these types of distortion; in other specimens only a single type occurred. In some specimens a single stool produced both normal and diseased heads, though this was unusual.

Many plants failed to develop heads and some apparently died early. The leaves of such plants were short, stiff, rather fleshy, and remained upright instead of curling downward normally (Fig. 1, F–G). On such plants the internodes usually were shortened and the tillers excessively numerous. Reddish-brown streaks and small ruptured areas were abundant on such leaves from the bases to the tips. Such lesions were much less common on plants producing deformed heads. These dwarfed, bunchy, nonfruiting plants were found scattered among those of normal height. Frequently, a stool was found with a number of culms of normal height and variously distorted. These stools also produced many bunchy, leafy branches from the base, some of which attained a height of only 4 or 5 inches.

Oospores were found in all diseased heads. They were closely invested with a heavy, persistent oogonial wall and were most abundant in the leaves, leaf-sheaths, rachis, rachilla, glumes, and other aboveground parts of the plants.

The disease was easily diagnosed in the oat plant on reaching the heading stage. By holding an infected leaf between the eye and the sun, with the aid of a hand lens, the oospores appeared as bright, translucent golden dots. They were usually grouped in rows between the vascular bundles, or in the bundles themselves, and were sometimes found scattered uniformly through the mesophyll tissues between the veins.

Symptoms on Wheat

The disease has been observed on a few wheat plants found in an infested field of oats. These plants were heading, much distorted, and sterile. The rachis was frequently so elongated as to widely separate the spikelets, especially toward the base, the head thereby losing its compact appearance. The rachis sometimes bent back on itself causing the head to form a circle or compact knot. The wheat found infected was bearded, the awns being much

curled and distorted. Such heads, therefore, were very conspicuous. Oospores were abundant in leaves, glumes, and awns.

The Causal Organism

The conidial stage of *Sclerospora macrospora* has not been found occurring in nature. The mycelium was intercellular and particularly abundant in the region of the vascular bundles. Haustoria, in the sense of those occurring commonly in the Peronosporaceae, were apparently lacking. Knobbed or wart-like outgrowths, however, were frequently observed, especially in the neighborhood of bast cells or pressing closely against the walls of nonpenetrated sieve tubes. From the accumulation of mycelium along the vascular bundles single strands were observed extending out between the cells of the parenchyma. Magnus (11) reported that *S. kriegeriana* (shown by Saccardo to be merely a synonym of *S. macrospora*) on *Phalaris arundinacea* L. produced hyphal branches that extended to the stomata where they gave rise to side outgrowths. These pushed out as short papillae. Magnus suggested that these outgrowths possibly represented rudimentary conidiophores, capable, under certain conditions, of developing further and becoming functional. These structures have not yet been observed on infected oat plants.

Oogonia and antheridia developed abundantly on the hyphae that were parallel to the vascular bundles. The antheridia developed on side branches in the vicinity of the oogonia. It was evident, however, that the resting spore, as observed in infected oat plants, consisted of the mature oospore closely invested in the thick, persistent oogonial wall. This wall frequently showed remains of the oogonial stalk cell, and, in young resting spores, remains of the antheridium sometimes may be observed attached at some point on the side or apex. When fully mature the oogonia were quite distinctive. The oogonial wall was generally smooth, though sometimes, when removed from the host plant, it exhibited roughened areas caused by fragments of the host cells against which it was closely appressed and adherent. Sometimes a portion of the outer oogonial wall exhibited knobs or projections which conformed closely to the outline of the pits in the walls of the bast fibers or phloem sieve tubes against which they were appressed. The pale-yellow oogonial wall usually very closely invested the single oospore, though narrow lumina frequently were observed between the two walls. Where there was a thickened place in the oogonial wall, as sometimes occurred, it seemed to be caused by a natural thickening; probably induced by unequal pressure or irregularity in the intercellular space in which it was formed. It was not caused by an overlapping or folding, as was the case for *Sclerospora graminicola* (Fig. 2, B).

The average thickness of the oogonial wall of 543 oospores from oats, wheat, corn, and barley was 4.2 μ. When measured along the long diameter of the oospore, the average thickness of the wall was 4.4 μ as compared with 4.1 μ for the short diameter. The reason for this difference was caused

by the partial persistence of the old oogonial stalk cell. In a few cases the thickness at this point was as much as 10 μ.

The oospores varied in shape from spherical to oblong. The walls, always smooth, varied in thickness from 2 to 4 μ, and lacked the yellow color of the oogonial wall. The oospore contents were yellowish-gray and densely granular. Sometimes lighter areas occurred in which small oil-like droplets were observed. In Table 1 are presented measurements of oospores from a

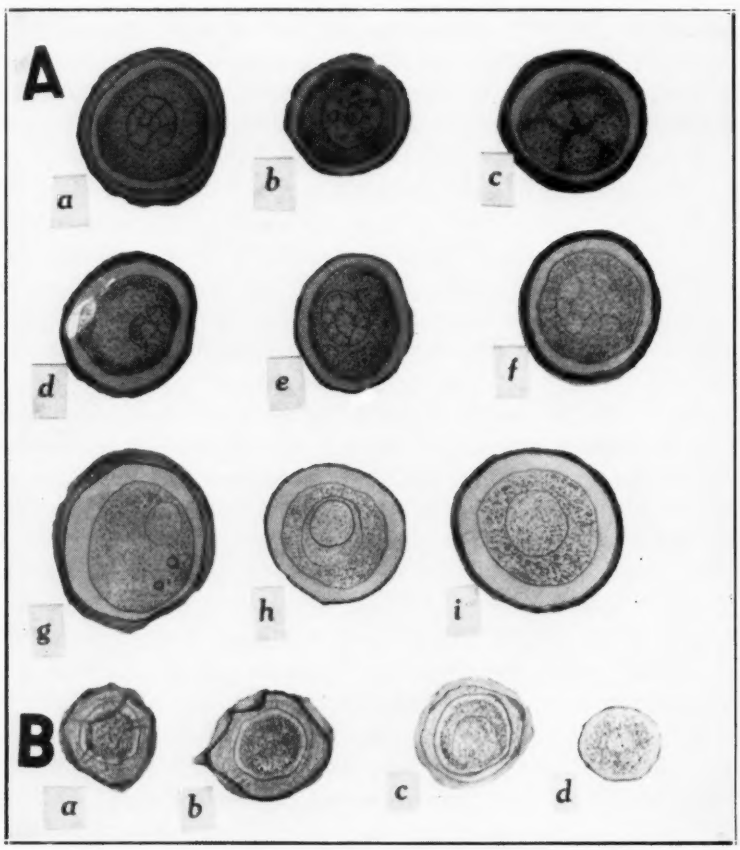

FIG. 2. A. Oospores of *Sclerospora macrospora* from *Avena sativa* L.; *a–c*, collected at Indianola, Mississippi; *b*, oogonial wall removed; *d–f*, collected at Bologna, Italy; *e*, oogonial wall removed; *g–i*, collected in New South Wales, Australia; *h*, oogonial wall removed. B. Oospores of *S. graminicola* from *Pennisetum typhoideum* L. Rich. collected at Poona, India; *d*, oogonial wall removed. Camera lucida drawings. ×375.

number of host plants from various localities. Only average diameters of oospores are considered significant, since observations have shown this to be the most constant factor. The enclosing oogonial wall was disregarded in all cases in making measurements, since its shape varied widely because of the variation in contour of the intercellular spaces in which the spores were formed. The number of measurements recorded varied for different collections, ranging from 900 on cheat grass from Tennessee and Kentucky to 50

on a number of others in which only a limited amount of material was available. Some of the measurements were made by the writers and some by W. H. Weston.

Two collections from barley are presented in the table, one from Cali-

TABLE 1.—*Comparative diameter of the oospores of Sclerospora macrospora and S. graminicola collected by various investigators in several localities*

Host	Locality	Collector	Number of oospores measured	Average diameter of oospores in microns	Range in microns	Mode in microns
		Sclerospora macrospora				
Oats	Mississippi	L. E. Miles	335	55.6	34.6–74.9	54–56
Oats	Yanco, New South Wales	R. J. Noble	100	58.6	40.6–73.8	54–56
Oats	Sydney, New South Wales	R. J. Noble	100	61.8	49.0–79.0	56–58
Wheat	Kentucky and Tennessee	W. H. Weston	612	62.5	38.2–88.2	62–64
Wheat	Delaware	J. F. Adams	168	60.9	50.9–78.0	60–62
Wheat	Assenciní, Italy	Cavara	50	60.1	45.0–72.0	62–64
Wheat	Piemonte, Italy	.....	50	65.6	43.2–68.4	54–56
Wheat	Samoggia, Italy	G. Goidanich	50	64.3	52.2–77.4	62–64
Wheat	Sardigna, Italy	Briosi	50	58.6	49.0–72.0	54–58
Wheat	Bologna, Italy	V. Peglion	100	53.6	45.0–64.8	54–56
Wheat	Padova, Italy	Saccardo	50	62.9	52.9–75.6	62–64
Barley	California	J. E. Coke	100	58.9	47.0–71.2	56–58
Barley	Bologna, Italy	V. Peglion	100	63.0	45.0–72.0	62–64
Corn	Bologna, Italy	V. Peglion	100	53.9	36.0–69.4	54–56
Rice	Taki-no-saki, Japan	G. Yamada	100	50.8	35.0–64.0	50–52
Rice	Morioka, Japan	G. Yamada	200	47.9	32.0–66.0	50–54
Avena fatua	Parkes, New South Wales	R. J. Noble	175	48.0	31.0–60.0	46–48
Bromus commutatus	Tennessee	W. H. Weston	900	60.1	32.0–84.0	62–64
Eragrostis major	Condobolin, New South Wales	R. J. Noble	50	62.3	56.1–72.7	62–64
Syntherisma sanguinale	Morioka, Japan	G. Yamada	100	44.9	30.3–55.4	44–46
		Sclerospora graminicola				
Pennisetum typhoideum	Poona, India	B. N. Uppal	200	30.4	21.6–39.6	32–34

fornia, collected by J. E. Coke, and one from Italy, collected by Vittorio Peglion. The Italian collection averaged larger both in average diameter and in mode than did the one from California. The total range, however, was very similar for both.

Only one collection from corn was measured. This was made by Peglion

in Bologna, Italy. It is worthy of note that the measurements on this host were practically identical with those on wheat, collected in the same locality in the same year by the same collector.

Two collections on rice from Japan, from two different localities, namely, Taki-no-saki and Morioka, were measured. These agreed quite closely but were smaller than the measurements from either oats, wheat, barley, or corn. Only one other collection of *Sclerospora macrospora* from Japan was available, and it was on a grass, *Syntherisma sanguinale,* collected by G. Yamada, in Morioka, Japan, in 1911. The spores in this instance were even smaller than on rice, and were the smallest observed. The average diameter of the oospores was only 44.9 μ, the range in diameter was from 30.3 to 55.4 μ, and the mode was 44 to 46 μ.

A collection of oospores on *Avena fatua,* a wild oat, collected near Parkes, New South Wales, by R. J. Noble in 1926, was only slightly larger, being about midway between the one on *Syntherisma sanguinale* and those of two collections on rice from Japan. It is interesting to note that a collection on *Eragrostis major* made at Condobolin, New South Wales, by R. J. Noble in 1936, had considerably larger spores, agreeing rather closely with the sizes of those on wheat and on cheat from Tennessee and Kentucky.

A collection of *Sclerospora graminicola* on *Pennisetum typhoideum* from Poona, India, collected by B. N. Uppal, in 1934, was measured and is presented in the table in order to show the much smaller spore sizes for the latter species as compared with *S. macrospora.* The average diameter of 200 spores of *S. graminicola* was only 30.4 μ, the range in diameter was from 21.6 to 39.6 μ, and the mode was from 32 to 34 μ. It will be noted that the average diameter of the oospore of this latter species was essentially the same as the diameter of the smallest oospore measured on *Syntherisma sanguinale,* which had the smallest spores of any collection of *S. macrospora* measured.

The average diameter of 335 oospores from oats collected in Mississippi in 1939 and 1940 was 55.6 μ, while 100 spores from a collection on the same host from Yanco, New South Wales, made by R. J. Noble, in 1924, averaged 58.6 μ and 100 spores from another oat collection from Sydney, Australia, averaged still larger, namely 61.8 μ. The mode for the average diameter measurements for the collections from Mississippi and for the one from New South Wales, Australia, was 54–56 μ, while that for the other collection from Australia was 2 μ larger.

The average diameter of 612 oospores from wheat from Tennessee and Kentucky, collected by W. H. Weston, was 62.5 μ; that of 168 oospores from wheat from Delaware, collected by J. F. Adams, was 60.9 μ; and the average diameter of 400 spores from 7 different collections from various points in Italy, made from different localities and in different years by different collectors, was 59.6 μ. The mode for the Tennessee and Kentucky collections was from 62 to 64 μ, while that from Italy was 58 to 61 μ.

These measurements tend to exhibit a very considerable degree of similar-

ity in spore sizes for all collections from the United States, Italy, and Australia, regardless of the host plant, except for the single collection on *Avena fatua* from New South Wales. This seems to fall into another group composed of the specimens on rice and *Syntherisma sanguinale* from Japan. It is unfortunate in this case that no collections of the fungus on rice from Italy were available for measurement. Table 2 presents a tabulation of

TABLE 2.—*Tabulation of frequency distribution of oospore size of Sclerospora macrospora on various host plants from several localities*

Range in diameter of oospores in microns	Oats		Wheat			Cheat	Rice		*Syntherisma sanguinale*	*Avena fatua*
	New South Wales	Mississippi	Kentucky & Tennessee	Delaware	Italy	Kentucky & Tennessee	Takinosaki, Japan	Morioka, Japan	Japan	New South Wales
	100 spores	335 spores	612 spores	168 spores	355 spores	900 spores	100 spores	200 spores	100 spores	175 spores
30–31.9	.....	.....	.....	.....	.....	.....	.....	.....	2	1
32–33.9	.....	.....	.....	.....	.....	2	.....	2	2	1
34–35.9	.....	1	.....	.....	.....	1	1	.....	5	1
36–37.9	.....	1	2	.....	.....	.....	.....	3	2	2
38–39.9	.....	1	1	.....	.....	3	.....	2	7	8
40–41.9	1	8	.....	.....	.....	6	1	4	10	11
42–43.9	2	4	3	.....	2	8	9	8	9	12
44–45.9	3	23	8	.....	4	6	13	14	15[a]	21
46–47.9	2	13	14	.....	10	27	16	22	13	34[a]
48–49.9	4	21	14	.....	10	42	15	29	13	27
50–51.9	7	18	16	2	22	64	18[a]	37[a]	12	23
52–53.9	4	20	30	14	18	50	12	21	8	10
54–55.9	15[a]	75[a]	37	18	37	52	7	15	3	8
56–57.9	8	29	42	28	36	81	4	13	.....	5
58–59.9	10	26	62	28	41	98	1	10	.....	4
60–61.9	10	29	64[a]	30[a]	45	103	2	2	.....	7
62–63.9	11	33	54	17	57[a]	105[a]	1	6	.....	.....
64–65.9	6	6	59	17	26	82	.....	.....	.....	.....
66–67.9	8	10	63	6	15	55	.....	.....	.....	.....
68–69.9	3	10	46	2	15	52	.....	.....	.....	.....
70–71.9	2	4	30	3	13	39	.....	.....	.....	.....
72–73.9	4	2	21	.....	1	25	.....	.....	.....	.....
74–75.9	.....	1	20	.....	2	9	.....	.....	.....	.....
76–77.9	.....	.....	12	1	1	5	.....	.....	.....	.....
78–79.9	.....	.....	5	.....	.....	2	.....	.....	.....	.....
80–81.9	.....	.....	4	.....	.....	4	.....	.....	.....	.....
82–83.9	.....	.....	2	.....	.....	2	.....	.....	.....	.....
84–85.9	.....	.....	2	.....	.....	1	.....	.....	.....	.....
86–87.9	.....	.....	2	.....	.....	.....	.....	.....	.....	.....

[a] Mode, or range within which the largest number of oospores were found.

frequency distribution of oospore diameters for several of the above collections and shows this grouping in a more graphic form.

DISSEMINATION AND PERSISTENCE OF THE PARASITE

Since a conidial stage has not been found, dissemination and persistence of the causal organism must occur most frequently through the resting

spores. There is a slight chance that the mycelium might reach the seed and lie dormant therein, although plants are usually so severely diseased as to produce no viable seeds. It seems improbable that wind is a very important factor in the dissemination of the oospores since, unlike *Sclerospora graminicola*, the invaded tissues are rarely shredded. It is possible that eroding soil, containing oospores, may be a means of distribution.

There is little doubt that the fungus persists in the soil of infested fields through the agency of the oospore, invested and protected as it is by the thick, hard, persistent oogonial wall. The writers have not yet observed the germination of the oospore, and this has been the experience of most others who have worked with this fungus, or for that matter with other species of the genus *Sclerospora*. Both laboratory and field observations, however, indicate that the oospores do undoubtedly germinate and bring about infection of the host plant.

Peglion (20, 21) reported germination of oospores of *Sclerospora macrospora* in 1914. The germination occurred by means of a large macroconidium produced on a short peduncle that issued through a split in the wall. The mature lemon-shape macroconidium bore a very prominent papilla and measured 75–80 × 55–60 μ. The contents of the conidium became differentiated into zoospores, though in rare instances the conidium germinated by means of a germ tube. Peyronel (23) reported similar results from a slightly different method. He observed germination of the oospores of *S. macrospora* by means of a conidium, or zoosporangium, and zoospores. This report is extremely interesting from a taxonomic as well as a practical viewpoint, since, if substantiated and confirmed as a general occurrence, it would necessitate removal of the species from the genus *Sclerospora* or, at least, a revision or redescription of the genus, since the present accepted method of germination for all members of the genus is by means of a germ tube issuing directly from the oospore.

Dissemination from one locality to another may occur through trash in the seed, consisting of leaf and glume fragments or other oosporic-bearing material, and by wind and erosion. Centrifugal separation has disclosed free oospores and minute fragments of oospore-bearing materials in seed lots that, from casual inspection, would have been considered well-cleaned. Moreover, some of the seed-like structures that replace the grain in the infested plant may very readily, and probably do, become mixed with the seed grain and might carry either spores or dormant mycelium and thereby establish an infestation in a new field. Further investigation is necessary to settle these points.

There are also other questions that require further elucidation. It has become rather generally accepted that a period of flooding of the host is a necessary condition to its infection through the oospores of *Sclerospora macrospora* (29). The writers, likewise, have found that the disease in Mississippi is much more frequent on land that has undergone a period of flooding. It has, however, been observed in numerous high and

well-drained fields where flooding could scarcely have been possible. It is true that in such cases infected plants were evenly and sparsely scattered and that in such fields the disease did not occur in epiphytotic proportions. Coke (according to Mackie) stated (10) that the disease nearly destroyed a crop of barley in California in a region where rainfall was scanty and where at least one irrigation was necessary to bring the crop to proper maturity.

DISCUSSION AND CONCLUSION

At present the downy mildew of wheat and oats (*Sclerospora macrospora*), as it occurs in Mississippi, is not of great economic importance. In isolated instances and restricted areas, however, where the fields are low-lying and subject to submergence, or where drainage is poor, the losses locally may be quite considerable. It is becoming a common practice in Mississippi to grow oats on the "buck shot" (clay) type of land, especially in the Delta. Since cotton acreage reduction practices have come into general use, the smaller number of acres devoted to cotton are being selected from the more loamy types of soil, where conditions permit, since such soil can be more readily prepared for early planting, thereby leaving the heavier and wetter areas for oats and similar crops. Such "buck shot" soil does not require complete submergence to become thoroughly saturated with water for considerable periods of time. The increasing tendency to utilize such practices may render more serious this disease of the oat crop, which is rapidly growing in importance in the particular area of the State in which the trouble occurs. With wider dispersal of the spores of the causative organism and with increasingly heavy soil infestation from repeated cropping with a susceptible crop, heavy and widespread losses may occur under certain seasonal conditions especially favorable to the pathogen.

SUMMARY

This report comprises the first record of *Sclerospora macrospora* (downy mildew) on oats in the United States and the first record of the disease in the State of Mississippi.

Symptoms of the disease on oats are described and compared with symptoms on wheat. Affected oat plants are characterized by stiff, curled, and fleshy leaves. The rachis is usually short and twisted and produces few spikelets, most of which are sterile. Many diseased plants fail to produce heads and apparently die early in the season.

Spores are produced in all parts of the plant, except the roots, and are much more abundant in the leaves and glumes.

The average diameter of 335 oospores from oats collected in Mississippi in 1939 and 1940 was 55.6 microns.

DEPARTMENT OF PLANT PATHOLOGY,
MISSISSIPPI AGRICULTURAL EXPERIMENT STATION,
STATE COLLEGE, MISS.

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A COMPARISON OF DIPLODIA NATALENSIS FROM STAINED WOOD AND OTHER SOURCES

A. F. VERRALL

(Accepted for publication March 2, 1942)

INTRODUCTION

One of the important staining fungi on logs and lumber in the Southern States has been referred to *Diplodia natalensis* Evans (1, 8). Morphologically similar fungi have been reported as pathogens on plants, including cotton (3), citrus (6), Honey Dew melon (10), and strawberry (5). Stevens (7) referred the citrus form, *D. natalensis*, and the cotton form, *D. gossypina* Cooke, to the same perfect stage *Physalospora rhodina* (Berk. and Curt.) Cooke, and stated that this fungus appears to have a wide distribution geographically and in its host relationships. Cross inoculations (2, 9, 10) have proved the wide range of pathogenicity of the pycnidial forms of *P. rhodina* from a large number of hosts. None of this inoculation work, however, was done with isolates from stained logs or lumber.

Little fruiting of *Diplodia natalensis* was observed on stained lumber and logs from which this fungus was isolated, and, in an earlier paper (8), the author suggested that the inoculum of *D. natalensis* causing stain in logs and lumber largely came from other sources. To test this contention, isolates of *D. natalensis*, or similar fungi, were secured from cotton bolls, a tung root, a pear stem, and a citrus fruit, to compare with the isolates secured from stained wood as to certain cultural characteristics, spore size, wood-staining ability, and pathogenicity on orange fruits, cotton bolls, and the stems of tung seedlings.

THE ISOLATES, THEIR CULTURAL CHARACTERISTICS

In table 1 are listed pertinent data on the source of the isolates used, together with data on certain cultural characteristics.

Although among the isolates there was considerable variation in general cultural appearance when grown on malt agar at room temperature (25°–30° C.), no constant differences between isolates from different host groups were evident. All grew rapidly, 15 to 20 mm. in radius per day, at room temperature.

Each isolate was grown in duplicate on malt agar at about 37° C. (35.6°–38.9° C.), and all were found to grow at this temperature (Table 1). Rapid growth at temperatures above 35° C. was one of the characteristics previously reported (8) for the wood-staining *Diplodia natalensis*. At this high temperature all the isolates, except 2 from wood (W 5 and 6), produced a pink coloration in the mat and agar instead of the usual grayish-black shades produced at room temperatures. Pink chromogenesis of agar at 37° C. has been described for some forms of *Physalospora rhodina* (7, 9).

Spore measurements (Table 1) were each based on 20 exuded, bicellular, colored spores formed on malt agar at 25 to 30° C. Although there was considerable variation in average spore size, there was little indication of significant differences between isolates from wood and from other sources, and all fell well within the range for average spore size of different isolates listed by others (7, 9). All the isolates were similar in having longitudinal striations in the cell walls of the dark, bicellular spores and in producing hyaline, unicellular spores preceding or in mixture with the 2-cell spores. The average spore size of the isolates from wood was somewhat larger than previously reported (8) for a larger number of isolates from wood.

TABLE 1.—*The isolates of Diplodia used—source, type, growth rate, and spore size*

Designation	Host from which isolated	Locality and date of isolation	Type of isolate	Growth in 24 hours at 37° C. in mm.	Average spore size in microns
C1	Cotton	Louisiana 1939[a]	Mass	5.5	26.3 × 14.0
C2	Cotton	Mississippi 1938[b]	Monoconidial	7.0	26.4 × 14.1
C3	Cotton	Alabama 1939[b]	Mass	8.0	25.3 × 12.2
C4	Cotton	So. Carolina 1939[b]	Mass	6.5	26.0 × 12.8
C5	Cotton	Virginia 1939[b]	Mass	5.5	25.1 × 12.6
Cit	Citrus	Florida[c]	?	7.0	28.1 × 14.4
P	Pear	Louisiana 1936[a]	?	4.0	30.4 × 14.8
T	Tung	Louisiana 1936[a]	?	7.0	24.2 × 12.7
W1	Pine log	Louisiana 1937	Monoconidial	7.5	26.5 × 13.4
W2	Pine lumber	Louisiana 1937	Monoconidial	7.0	26.4 × 13.5
W3	Pine lumber	Louisiana 1937	Monoconidial	8.5	29.8 × 14.3
W4	Pine log	Louisiana 1937	Monoconidial	7.0	28.0 × 12.7
W5	Yel. poplar lumber	Louisiana 1937	Monoconidial	4.0	26.2 × 15.0
W6	Yel. poplar log	Louisiana 1937	Monoconidial	7.0	30.0 × 15.4
W7	Magnolia log	Louisiana 1937	Monoconidial	7.0	26.2 × 11.8
W8	Sweet-gum lumber	Louisiana 1937	Monoconidial	2.5	26.9 × 14.4

[a] Secured from Louisiana State University.
[b] Secured from the South Carolina Agricultural Experiment Station, Clemson.
[c] Secured from the Florida Agricultural Experiment Station, Citrus Experiment Station, Lake Alfred.

Due to the difficulty of separating *Physalospora rhodina*, the perfect stage of *Diplodia natalensis*, from certain other species of *Physalospora* on the basis of the morphology of the pycnidial forms and host relationships (7, 9), it is not certain that all the isolates from stained wood, that have the general characteristics of *D. natalensis*, are in reality *P. rhodina*. No perfect stages of the isolates used in this study were observed in culture nor have any been observed on stained logs and lumber. For convenience, the wood-staining forms are called *Diplodia natalensis* but with the realization that if complete life histories were known some isolates might have perfect stages other than *P. rhodina*.

WOOD-STAINING ABILITY

To test their wood-staining ability, various isolates were grown on pine and sweet-gum wood. Discs of sapwood, approximately 2½ inches in diam-

eter and ½ inch thick, were cut from the stems of small living trees and after removal of the bark were immersed for 10 seconds in boiling water, and then placed in sterile Petri dishes. Three discs of each species of wood were inoculated with actively growing mycelium of each of 13 isolates and incubated at approximately 25° C. for two weeks. The isolates used and the results of the inoculations are listed in table 2.

After an incubation period of two weeks, all the inoculated pine discs were completely stained and all the inoculated sweet-gum discs were stained in the outer portions, although the centers were still stain-free. The uninoculated discs of sweet gum and pine remained bright and stain-free, except for chemical brown stain found under the surface of the sweet-gum wood. This chemical stain was distinct in color and distribution from the fungus stain of the inoculated wood. There was no discernible difference in color or extent of stain caused by isolates from stained wood and other sources.

DECAY OF CITRUS FRUITS

Diplodia natalensis has been reported as causing several citrus diseases, including an important fruit decay (6). All the isolates from cotton, citrus, tung, and pear and six from wood (W 2, 3, 4, 5, 6, and 7) were tested for their ability to decay citrus fruit.

Oranges, 2½ to 3 inches in diameter and firm, were secured on the open market. The stem abscission scars were lightly flamed with alcohol and punctured with a sterile scalpel. A small block of mycelium bearing agar was placed over the scars, and the fruits were placed in an incubator at approximately 25° C. Two oranges were inoculated with each isolate and 6 with sterile agar as controls.

Five to 8 days after inoculation most of the oranges were soft and watery from decomposition of the white inner portions and the membranes between segments. The pathogen was reisolated from the center of each orange inoculated with isolates P, T, C 1, C 2, C 4, C 5, W 2, W 4, and W 5, and from 1 of 2 oranges inoculated with each of W 3, W 7, and Cit. The second orange of these latter pairs, both inoculated with C 3, and all controls, showed no decomposition and remained sterile. Since the inoculum blocks were not protected from drying, it seems probable that the few inoculations not resulting in infection were due largely to rapid drying of the inoculum blocks. In another series of inoculations with the isolates that had given some or no infection, the inoculated fruits were placed in closed glass containers for 8 hours prior to placing in the larger incubator previously used. All of these inoculations gave positive results (Table 2).

PATHOGENICITY ON COTTON BOLLS

Diplodia gossypina is one of the fungi causing boll rot of cotton (3). As was pointed out in the introduction, this fungus is considered identical with *D. natalensis*.

Bolls one inch or more in length and growing on plants in the open

were inoculated on several dates between June 15 and July 15, 1941. Inoculation was accomplished by cutting a small incision through the wall of the boll, inserting a small piece of mycelium grown on malt agar, and pressing the wound together. In the controls the incision was made but no inoculum was introduced. The isolates used, the number of bolls inoculated, and the number of bolls showing decomposition are given in table 2.

Most of the inoculated bolls exhibited some decomposition in 2 to 5 days. Usually the entire boll turned brown to black on the outside, and the interior was disintegrating rapidly 4 to 5 days after inoculation. Isolates W 8, P, T, and Cit, however, caused little discoloration or decomposition of the boll wall when inoculated into larger bolls, even when decomposition of the interior was advanced. Four to 5 days after inoculation the pathogen was reisolated from the interior of each inoculated boll, except from 1 boll inoculated with Cit and 1 with T. In these cases very little decomposition occurred and only bacteria were reisolated. Although each isolate used caused almost complete disintegration of at least one inoculated boll, the isolates from cotton and isolates W 1 and W 2 distinctly caused more rapid decomposition than did the rest. None of the controls developed black-boll rot symptoms, although a slight browning and

TABLE 2.—*Results obtained by inoculating sapwood discs, orange fruits, stubs and shoots of tung seedlings, and cotton bolls with various isolates of Diplodia natalensis*

Isolate	Ratio: Number of infections/Number of inoculations[a]						
	Stain of sapwood discs		Decomposition of orange fruits		Cankers on tung seedlings[b]		Cotton boll rot
	Sweet gum	Pine	Test 1	Test 2	Stub inoculations	New-shoot inoculations	
C 1	3/3	3/3	2/2	.....	0/2	0/3	5/5
C 2	3/3	3/3	2/2	.....	0/4	0/3	6/6
C 3	3/3	3/3	0/2	3/3[a]	.....	.....	6/6
C 4	3/3	3/3	2/2	.....	.....	.....	.....
C 5	3/3	3/3	2/2	.....	0/3	0/3	.....
Cit	3/3	3/3	1/2	2/2	0/5	0/4	7/8
P	3/3	3/3	2/2	.....	0/6	0/6	5/5
T	3/3	3/3	2/2	.....	0/5	0/3	6/7
W 1	3/3	3/3	.....	.....	0/4	0/3	5/5
W 2	3/3	3/3	2/2	.....	.....	.....	5/5
W 3	.....	.....	1/2	2/2	0/3	0/4	5/5
W 4	.....	.....	2/2	.....	.....	.....	.....
W 5	3/3	3/3	2/2	.....	1/4	0/4	5/5
W 6	3/3	3/3	2/2	2/2	.....	.....	.....
W 7	3/3	3/3	1/2	2/2	.....	.....	.....
W 8	.....	.....	.....	.....	0/4	0/3	5/5
Checks	0/8[c]	0/6	0/6	0/2	0/4	0/5	0/12

[a] The numerator indicates the number of infections and the denominator the total number of inoculations.

[b] *D. natalensis* persisted as a saprophyte on the dead stubs beyond the uppermost bud and at points of inoculation on new shoots where temporary cankers were calloused-off.

[c] Chemical brown stain occurred in both inoculated and noninoculated sweet gum discs. This was distinct from the fungus stain in the inoculated discs.

softening occurred at the incision point. Bacteria were isolated from the browned tissue.

PATHOGENICITY ON TUNG TREES

Diplodia natalensis has been reported as a pathogen on tung (4, p. 224; 9), although it is apparently of little importance on this host.

One hundred seedlings,[1] 9 months old and with the tops removed 6 to 8 inches above ground, were transplanted during the dormant season. One-half of these were lightly fertilized with a complete fertilizer (4–8–4). At the start of shoot growth 40 trees were inoculated on the stub above the sprouts by placing mycelium-bearing agar into an incision through the bark. The isolates used and the number of plants inoculated with each are listed in table 2. Sterile agar was applied on 4 as checks. The wounds were wrapped with wax paper, which was removed a week later. After the sprouts were approximately 0.5 inch in diameter, similar inoculations were made into the shoot growth on 36 remaining plants, with sterile agar on 5 as checks.

With the stub inoculations, *Diplodia natalensis* spread down to the region of the sprout, fruiting copiously in the bark, but there was no indication (7 months after inoculation) that it could spread into the sprout or below the sprout in the stub except in one instance in which a weak plant was killed back to the ground line (W 5). A vigorous root sprout was formed 6 weeks after the inoculation had been made. The checks likewise died back to the sprouts, but more slowly than did the inoculated plants.

In the sprout-inoculated series the incision on the 5 checks healed completely in 2 weeks, while black cankers formed at all incisions into which *Diplodia natalensis* had been inoculated. One month after inoculation strong callous growth had formed at the margins of all cankers preventing further spread of the fungus. Five months after inoculation all inoculated shoots were making vigorous growth and the cankers were almost healed over. Apparently the isolates used, including that originally from tung, are but slightly pathogenic on tung shoots.

SUMMARY AND CONCLUSIONS

Certain comparisons were made among isolates of *Diplodia natalensis* secured from stained lumber or logs and the same or similar *Diplodia* species from cotton, citrus, pear, and tung plants.

There was considerable variation among the isolates used in rate of growth at 37° C., spore size, and general cultural appearance. However, in no cultural characteristic studied did the isolates from stained lumber or logs differ materially from isolates from other sources.

All of 13 isolates tested on pine and sweet gum sapwood proved to be vigorous stainers of these species.

The 14 isolates tested on citrus fruit caused rapid decay. All of the 11 isolates tested on cotton caused black boll rot. None of the 10 isolates

[1] Furnished by the U. S. Field Laboratory for Tung Investigations, Bogalusa, La.

inoculated into small tung trees was sufficiently pathogenic to cause more than small temporary cankers that soon calloused over.

From these studies it seems that the *Diplodia natalensis* causing stain in lumber and logs is, at least from a practical point of view, the same fungus as the isolates secured from several plants. Little fruiting of *D. natalensis* has been observed on stained logs and lumber from which this fungus was isolated. Because of the similarities among isolates of this fungus from stained wood and other sources, it seems probable that some of the inoculum inducing wood stain in logs and lumber is derived from spores produced on such plants as cotton.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH THE SOUTHERN FOREST EXPERIMENT STATION,
NEW ORLEANS, LA.

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THE INFLUENCE OF VITAMIN B_1 ON THE DEVELOPMENT OF CANTALOUPE POWDERY MILDEW

DEAN E. PRYOR[1]

(Accepted for publication March 3, 1942)

INTRODUCTION

Within the last 6 years the effect of vitamin B_1 (thiamin hydrochloride) on plants in their entirety and on different plant organs has been studied considerably. Robbins and Kavanagh (9) point out that there have been conflicting reports regarding the vitamin B_1 stimulation of flowering plants, but that many other organisms have been shown to be heterotrophic, autotrophic, or intermediate with regard to their thiamin requirements. Since these investigators have summarized most of the important literature to date, only papers pertinent to the present investigation will be mentioned. Henry[2] found the vitamin B_1 concentration higher in crown-gall tissue than in stem tissue. This increase was evident shortly after inoculation and before macroscopic galls appeared. Stem tips, however, contained more thiamin than did the galls. When plants were grown at temperatures above the maximum for gall development, the vitamin B_1 content was about the same as that in plants grown at temperatures that permitted galls to appear, indicating no deficiency of vitamin B_1 at the higher temperatures. He concluded that this vitamin does not influence crown-gall development more than any necessary food or growth factor.

There seems to be no published information concerning the influence of thiamin on the host-parasite complex. The present paper summarizes results of an investigation on the development of race 2 of cantaloupe powdery mildew (*Erysiphe cichoracearum* DC.) as affected by thiamin. An abstract of preliminary results has already been published (8).

METHODS AND MATERIALS

Two strains of melons were employed in this study, Powdery Mildew Resistant Cantaloupe No. 45, the common commercial melon of the Imperial Valley, resistant to race 1 of the fungus (5) but susceptible to race 2 (6), and strain 28949, a line completely resistant to race 1 but characterized by necrotic spotting when artificially inoculated with race 2. Macroscopically visible mycelium has never been noticed on plants of the latter strain, either in the greenhouse or in the field. Under the conditions of the present experiments strain 28949 seemed somewhat less vigorous than No. 45. Here-

[1] Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The author wishes to thank Dr. V. R. Boswell of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful advice on the statistical phases of this study.

[2] Henry, Berch W. The relation of vitamin B_1 to crown-gall development. Unpub. Ph.D. thesis deposited in library of Univ. of Wis., Madison, Wis. 1941.

after, these two strains of cantaloupe may be referred to as the susceptible variety (No. 45), and the resistant variety (strain 28949).

The stock culture of *Erysiphe cichoracearum* used in these tests was obtained from No. 45 plants naturally infected in the Imperial Valley and was, therefore, probably composed entirely of race 2. The fungus was maintained on convenient susceptible plants and occasionally was reinoculated on No. 45 to make certain its pathogenicity had not changed. Since stock plants were susceptible to both mildew races, it is possible that race 1 may have been carried along as a contaminant. No differential hosts have been discovered that are susceptible to race 1 but not to race 2; consequently, it is difficult to recover race 1 from a mixture of the two. Variance analyses (10) were performed on all experimental data.

Soil Experiments

Effect of Thiamin on Disease Development. These experiments were carried out in a greenhouse maintained at a night temperature of about 65° F. and a maximum day temperature generally of 80° to 85° F. but occasionally going as high as 90° or 95° F. Cantaloupe seeds were sown directly in 6 in. pots, and, when cotyledons appeared, the plants were thinned to 5 per pot. Two pots receiving the same treatment were placed side by side and replicated 3 times. Applications of thiamin at the rate of 200 cc. per pot in concentrations of 0.00, 0.01, 0.10, 1.00, and 10.00 p.p.m. were made twice weekly, starting immediately after thinning. Thus in one planting there were 150 plants of each variety. The solutions were made up in tap water not more than an hour before they were used. Supplementary irrigation with tap water was given when required. During the 4 to 6 weeks that elapsed from the time seed was planted until disease notes were taken, 5 to 7 applications of thiamin were made.

As soon as the first true leaves of resistant plants unfolded, they were placed in a glass-sash chamber, accommodating 40 6-in. pots. The plants were then inoculated by blowing conidia from heavily infected leaves into the chamber and allowing them to settle. Blowing the spores from the leaves seemed to result in less clumping and a more uniform distribution of the conidia than did transferring by contact or dusting. The plants were inoculated in the morning and removed to the greenhouse the following morning. Repeated tests of a full chamber of susceptible plants proved the method to be satisfactory in obtaining a uniform, severe infection of the upper leaf surfaces. The following disease index was employed for the resistant variety: Necrosis or collapse of 0 to 20 per cent of the first true leaf was rated as 0; 21 to 40 per cent as 1; 41 to 60 per cent as 2; 61 to 80 per cent as 3; 81 to 100 per cent as 4. Except for one experiment the susceptible variety No. 45 was exposed only to greenhouse infection, and colonies were counted just before they fused together to become indistinguishable. This procedure was followed because it was found that artificial inoculation of this variety produced such severe disease effects that it was impossible to make colony

counts. Since the disease appeared in maximum degree more quickly on plants of the susceptible variety notes were taken on them sooner than on those of the resistant strain.

Effect of Thiamin on Growth. Immediately after disease notes were taken on the resistant variety, measurements of height and green weight of tops of both lines were made. This was done usually in the morning and always soon after the pots were watered. Measurements were never made when any of the plants showed signs of wilting.

Since it is practically impossible to maintain susceptible plants free from mildew in a greenhouse used for mildew tests, disease-free melons were grown in another greenhouse. The only one available was unheated and had a night temperature of 50° to 60° F. and day temperatures similar to those of the heated house. Except for the plants being free from disease and growing under a slightly different environment, the experiments with mildew-free plants were duplicates of the inoculated series in the heated house. Growth measurements were made at approximately the same stage of plant development as for the inoculated series.

Dish Experiments

Thiamin Added to Sucrose Solution upon which Inoculated Excised Leaves Were Maintained. Mildew-free plants of both the resistant and susceptible cantaloupe varieties were grown in the unheated greenhouse described above. When 2 to 3 leaves were present, the first and second leaves of approximately uniform size were excised, inoculated by brushing dry spores over the surface, and placed in Petri dishes to which 15 cc. of a sucrose solution and various amounts of thiamin were added. The sugar solution was composed of 100 g. of sucrose per liter of distilled water. Thiamin solutions were made up in such concentrations that a maximum of 1 cc. was required per dish to supply amounts of 1, 10, 100, or 1000 gamma. The dishes were incubated at room temperature. When the first macroscopic sign of mycelium appeared, the colonies were measured to the nearest millimeter and measurements were continued every day until 1 leaf in the series was completely covered with mycelium. There were 10 leaves of each cantaloupe variety for each concentration of thiamin and for the control, making 50 leaves for each strain in each experiment. The treatments were randomized and replicated and the resulting data subjected to statistical analysis.

Thiamin Added to Soil Supporting Mildew-free Plants from which Leaves Were Excised, Inoculated, and Maintained on a Sucrose Solution. First and second leaves from mildew-free plants as described in the section on effect of thiamin on growth were excised after growth measurements were taken. They were then inoculated by the brush method and placed in Petri dishes to which had been added 15 cc. of the sucrose solution described above. Colonies were measured as in the case of the previously mentioned dish cultures. These leaves thus received no external source of thiamin after being excised from the thiamin-treated plants.

EXPERIMENTAL RESULTS

Soil Experiments

Effect of Thiamin on Disease Development. The severity on the susceptible variety and on the resistant strain at about the stage when disease notes were taken is shown in figure 1. Table 1 expresses the means of 4 experiments with 30 susceptible and 30 resistant plants for each of the 5 treatments in each experiment. With the susceptible variety soil applications of thiamin solution at concentrations of 0.01 and 1.00 p.p.m. significantly

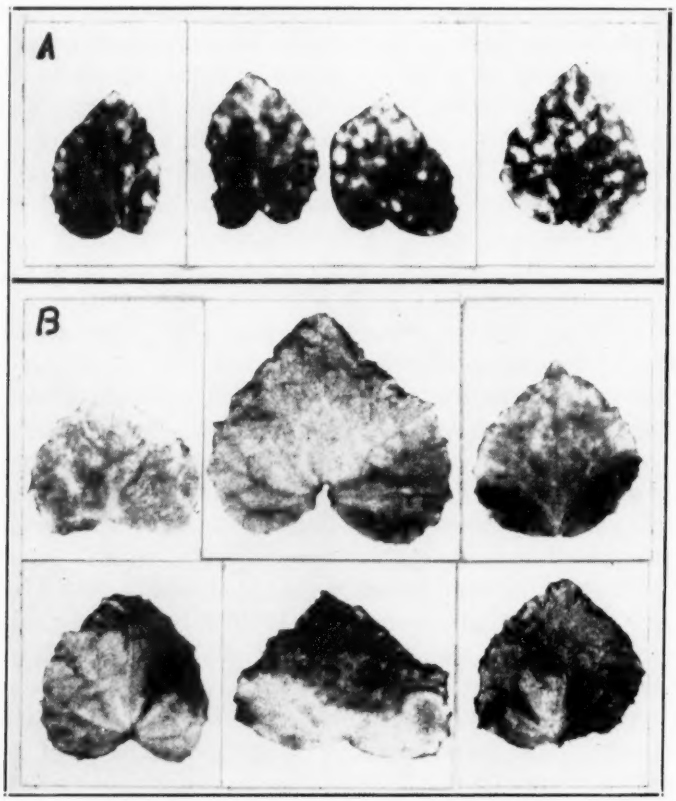

FIG. 1. Powdery mildew on cantaloupe leaves at about the stage when disease notes were taken. A. Leaves of the susceptible variety showing mildew colonies. B. Leaves of the resistant strain illustrating necrosis.

increased mildew growth over the control, but the difference between the 4 amounts of thiamin were not statistically significant. The mildew increase caused by 0.10 p.p.m. is barely under the value required for significance at the 5 per cent level. The numbers of colonies on the treated plants ranged from 1.3 to 1.5 times the control. With the resistant melon strain the results of the thiamin treatments were more striking, the amount of disease being increased 2.1 to 2.8 times for the several concentrations used, and all of these differences were significantly greater than the control. There were, however, no statistically significant differences between concentrations.

TABLE 1.—*Powdery-mildew development on cantaloupe plants grown in soil to which various concentrations of thiamin solution were added*

Melon variety	Total[a] number of plants	Soil watered with thiamin solution at concentration of					Least signif. mean diff. at 5% level	Least signif. mean diff. at 1% level
		0.00 p.p.m.	0.01 p.p.m.	0.10 p.p.m.	1.00 p.p.m.	10.00 p.p.m.		
Susc.	600	28.98[b]	39.65	38.95	43.55	37.34	9.98	13.11
Res.	600	0.72[c]	1.53	1.58	2.01	1.58	0.77	1.02

[a] Four experiments with 30 susceptible and 30 resistant plants for each of the 5 treatments in each experiment.

[b] Mean number of colonies counted on first true leaf of the susceptible No. 45 variety.

[c] Mean disease index based on condition of the first true leaf of resistant strain No. 28949: 0 = necrosis of up to 20% of the leaf blade; 1 = 21 to 40%; 2 = 41 to 60%; 3 = 61 to 80%; and 4 = 81 to 100% necrosis.

TABLE 2.—*Optimum concentration of thiamin for powdery-mildew development on susceptible and resistant cantaloupe plants in different experiments and different blocks in these experiments, based on averages for each concentration of solution applied to the soil*

Melon variety	Experiment number[a]	Optimum[b] concentration for entire expt.	Block	Optimum[b] concentration for block shown
		p.p.m.		*p.p.m.*
Susc.[c]	I	1.00	A	0.00
			B	1.00
			C	0.01
	II	0.10	A	0.10
			B	0.10
			C	0.01
	III	1.00	A	0.01
			B	0.10
			C	0.01
	IV	1.00	A	1.00
			B	1.00
			C	1.00
Res.[d]	I	1.00	A	0.01
			B	1.00
			C	1.00
	II	0.10	A	1.00
			B	0.00
			C	0.10
	III	1.00	A	1.00
			B	1.00
			C	0.10
	IV	1.00	A	1.00
			B	10.00
			C	10.00

[a] Even though experiments with susceptible and resistant varieties have the same Roman numeral designation, it is not to be construed that they were performed at the same time.

[b] Concentration resulting in greatest average development of powdery mildew.

[c] Cantaloupe No. 45 susceptible to race 2 of the pathogen.

[d] Cantaloupe strain No. 28949 resistant to race 2 of the fungus.

These averages, however, do not tell the whole story. As would be expected, the amount of disease on individual plants varied considerably, especially with the susceptible variety, which was infected by natural means in the greenhouse. This variation extended not only to blocks but to experiments. From table 1 it is apparent that 1.00 p.p.m. of thiamin tended to be the most favorable concentration for mildew development on both the resistant and the susceptible varieties. Table 2 shows the indicated optimum for the different experiments and blocks which contributed the data summarized in table 1. In experiment III with the susceptible melon variety, the optimum concentrations for blocks were 0.01, 0.10, and 0.01 p.p.m., respectively, whereas the optimum concentration for the experiment was 1.00 p.p.m. This apparent discrepancy resulted from the fact that the 0.10 p.p.m. concentration in Blocks A and C and the 0.01 p.p.m. concentration in Block B

TABLE 3.—*The average green weight and height of tops from diseased and healthy cantaloupe plants grown in soil watered with solutions of thiamin*

Plant condition	Growth measurement	Melon[a] variety	Total no. of plants	Soil watered with thiamin solution at concentration of				
				0.00 p.p.m.	0.01 p.p.m.	0.10 p.p.m.	1.00 p.p.m.	10.00 p.p.m.
Diseased	Weight in g.	Susc.[b]	300	3.32	3.99	3.01	3.46	3.22
		Susc.[c]	150	1.24	1.32	0.98	0.85	0.85
		Res.[c]	450	2.30	1.93	2.40	2.65	2.10
	Height in cm.	Susc.[b]	300	14.16	14.89	13.56	14.10	14.00
		Susc.[c]	150	9.68	9.52	9.36	8.78	8.31
		Res.[c]	450	12.10	10.95	12.35	12.90	11.39
Healthy	Weight in g.	Susc.	450	3.28	2.94	2.92	3.27	3.25
		Res.	450	2.26	2.77	2.19	2.43	2.52
	Height in cm.	Susc.	450	12.06	11.22	11.08	12.06	11.72
		Res.	450	10.01	11.20	9.79	10.80	10.80

[a] Susc. = Cantaloupe No. 45 susceptible to race 2 of the fungus.
Res. = Cantaloupe strain No. 28949 resistant to race 2 of the pathogen.
[b] Exposed only to natural spread of powdery mildew in the greenhouse.
[c] Artificially inoculated with powdery mildew.

caused less mildew development than the control, while the 1.00 p.p.m. concentration in Blocks A and B considerably increased the disease over the control, thus bringing the mean of this last concentration for all blocks above that of the other concentrations.

The deviation was so great that a few experiments analyzed separately showed no statistically significant stimulation. Nevertheless, when all of the experiments were combined and analyzed, the general effect of treatment was found to be significant. Since these particular experiments were in progress from January to June, it is not surprising that a given type experiment or block in that experiment exhibits different optima when performed at different times of the year under ordinary greenhouse conditions.

Effect of Thiamin on Growth. There was considerable variation in

height and weight of plants within treatments, blocks, and experiments. Table 3 shows the means of these values for treatments in all the experiments. While it appears that in most comparisons certain treatments were different from the control, none was significant when the "treatment" variance was compared with "treatment × experiment" variance as error. This was true whether treatment variances were determined as a whole or by single degrees of freedom. Individual experiments also were analyzed statistically, and in all but two there were no significant growth variations. In one experiment with mildew-free plants of the resistant variety, the green weight of plants treated with 0.01 p.p.m. concentration of thiamin was significantly greater than that of similar plants treated with 1.00 or 10.00 p.p.m. concentrations, but none of the thiamin-treated plants differed significantly from the control. In one trial with inoculated plants of the resistant variety, all of the plants treated with thiamin had significantly lower green weight than the control.

On a percentage basis, the growth increases were much smaller than those reported by Bonner and Greene (2, 3), but are not materially different from those reported by other workers (9). Robbins and Kavanagh (9) summarize the situation by saying "It appears that applications of thiamin to intact higher plants is without benefit or the conditions under which it is beneficial are ill-defined."

Dish Experiments

In contrast to the greenhouse trials, there sometimes appeared in the dish cultures a small amount of mycelium on leaves of the resistant strain, but, because of complicating factors, the results on this strain are not included. Areas of discoloration soon appeared in the resistant leaves, but it was difficult to distinguish whether the necrotic spots were caused by powdery mildew, senescence, or contaminating organisms. No necrosis similar to that appearing in the greenhouse was observed.

Thiamin Added to Sucrose Solution upon which Inoculated Leaves Were Maintained. Table 4 gives the results from 3 experiments with leaves of the

TABLE 4.—*Average area of powdery-mildew mycelium parasitizing excised susceptible leaves maintained on a sucrose solution to which various amounts of thiamin were added*

Days from inoculation	Total number of leaves[a]	Quantity of thiamin in 15 cc. of sucrose solution					Least signif. mean diff. at 5% level	Least signif. mean diff. at 1% level
		gamma 0	gamma 1	gamma 10	gamma 100	gamma 1000		
		area in sq. mm.	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*		
2	150	0.53	6.37	15.83	1.83	4.23	17.2	23.2
3	150	33.37	59.07	47.53	27.70	50.60	46.5	61.6
4	150	166.4	189.9	155.3	89.4	150.2	126.0	167.0
5	150	459.6	516.3	453.5	267.7	358.0	199.0	264.0

[a] Ten leaves per treatment in each of 3 experiments.

susceptible variety. Although treatments appear to have produced some large differences at 2 days in the means for the total of 3 tests, they are not significant. At 3 and 4 days all lots were quite similar. There was a barely significant difference between 1 and 100 gamma at 5 days, but neither of these values differed significantly from the control or any other concentration. Here again, behavior among experiments was highly variable. In the first test, 2 days after inoculation, 10 gamma produced enormously larger growth than any other treatment by significant odds. In the third test this treatment was exceeded by 1 gamma and in the second test 1 gamma showed no growth at 2 days. Such erratic results indicate the need for more refined and dependable methods for studying the effects of thiamin by this approach. The amount of vitamin B_1 in the leaves at the time they are excised may also contribute considerably to the variability. While it appears that thiamin added to the solution upon which leaves are maintained may stimulate growth of mildew colonies, the evidence is not conclusive.

TABLE 5.—*Average area of powdery-mildew mycelium on excised leaves from susceptible cantaloupe plants grown in soil that had been watered with various concentrations of thiamin solution*

Days from inoculation	Total number of leaves[b]	Soil watered with thiamin solution at concentration of					Least signif. mean diff. at 5% level	Least signif. mean diff. at 1% level
		0.00 p.p.m	0.01 p.p.m	0.10 p.p.m	1.00 p.p.m	10.00 p.p.m		
		area in sq. mm.	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*		
3–4[a]	100	3.30	12.30	8.65	9.00	7.20	8.54	11.6
4–5	100	49.5	115.2	55.2	65.3	60.9	78.4	105.0
5–6	100	142.0	308.3	195.4	228.9	161.5	97.8	131.0
6–7	100	340.5	556.0	468.3	453.8	365.1	168.0	226.0

[a] First measurements taken on the day mycelium became macroscopically visible, the incubation period requiring 3 days in some experiments and 4 days in others.

[b] Ten leaves per treatment in each of 2 experiments.

Thiamin Added to Soil Supporting Plants from which Leaves Were Excised, Inoculated, and Maintained on a Sucrose Solution. During 3 to 7 days after inoculation 3 out of the 4 observations showed a significant increase in mildew growth on leaves from plants watered with 0.01 p.p.m. thiamin solution as compared with that on the control plants (Table 5). No other treatment gave results significantly different from the control at any time after inoculation, but the 0.01 p.p.m. treatment resulted in significantly greater mildew growth than some of the other concentrations at 5 to 6 and at 6 to 7 days. It thus appears that a low concentration (0.01 p.p.m.) of thiamin solution tends to stimulate mildew growth under the conditions described. Although only 2 tests were involved, the results may be accepted with some assurance since more elaborate tests with infected plants growing in treated soil have shown that 0.01 p.p.m. significantly increased mildew.

DISCUSSION

Recently a dust containing vitamin B_1 was recommended for the control of cantaloupe powdery mildew in commercial melon fields, the assumption being that the supposedly more vigorous plants resulting from this treatment are resistant to mildew. A preliminary report (8) pointed out that thiamin could not be used effectively for this purpose and that in some cases it actually seemed to stimulate fungus growth. The present work shows that there was a statistically significant increase in parasitic activity due to thiamin, although the conditions under which it occurred were somewhat different from those indicated in the earlier work.

Obligate plant parasites in general seem to develop best when their host is growing most vigorously (13). There is, however, some indication that wheat mildew may be very active, even though the host be not in the best vegetative condition (11). In only one experiment with cantaloupe mildew did thiamin significantly affect growth of parasitized plants. In this case the green weight of thiamin-treated plants of the resistant variety was decreased and mildew was increased by significant odds. The lump analysis of all soil-treatment experiments shows that the growth of *Erysiphe cichoracearum* on the plants was increased significantly, while their height and green weight were unaffected by such use of thiamin. For a longer growth period the greater amount of mildew might have been accompanied by depressed growth of the host.

Since *Erysiphe cichoracearum* has not been cultured in the absence of the living host, the effect of nutrients or hormones on mycelial development are, at present, best studied by indirect methods. Results from an investigation employing such a technique can be accepted only with certain qualifications. Some of the difficulties have been enumerated in connection with studies of another fungus parasite (*Plasmodiophora brassicae* Wor.), which likewise has not been grown *in vitro* (7).

Although thiamin, when supplied to the host plant, tended to promote powdery-mildew development, it is not possible from the present data to be sure that thiamin as such directly stimulated growth of the fungus. Indirect evidence from other lines of work, however, gives some indication that thiamin itself may cause increased parasitic activity. Burkholder and McVeigh (4) found that the vitamin B_1 content of maize leaves varied directly with the nitrogen supply but, inversely, with the phosphorus in the nutrient solution. Turner (12) found that barley and corn, high in nitrogen, were readily attacked by mildew. Wheat inoculated with powdery mildew (*Erysiphe graminis* DC.) and supplied with various molecular proportions of salts was found to be most susuceptible when growing in a solution containing 5 per cent of KH_2PO_4, 47.5 per cent $Ca(NO_3)_2$, and 47.5 per cent $MgSO_4$ and least susceptible in one with 90 per cent of KH_2PO_4, 5 per cent $Ca(NO_3)_2$, and 5 per cent $MgSO_4$ (11). Wingard (13) pointed out that many diseases, particularly those caused by obligate parasites, are increased

by high nitrogen and decreased by high phosphorus as nutrients. Thus conditions of mineral nutrition that increase thiamin content have in many cases also increased development of certain fungus diseases. However, light, which also influences the quantity of vitamin B_1 in plants (1), may be a complicating factor in this connection. From the data presented in this paper, we can, therefore, conclude that growth of *Erysiphe cichoracearum* on cantaloupes is either directly or indirectly increased by thiamin.

SUMMARY

Of the two melon strains employed in this study, Powdery Mildew Resistant Cantaloupe No. 45, highly resistant to race 1, was readily attacked by race 2 of *Erysiphe cichoracearum;* while Cantaloupe Strain 28949, after artificial inoculation with race 2, produced necrotic spots, generally without macroscopically visible mycelium. When vitamin B_1 (thiamin hydrochloride solution) was added in various concentrations to soil in which these infected cantaloupe strains were growing, the average number of powdery-mildew colonies on the susceptible variety (No. 45) for the several treatments ranged from 1.3 to 1.5 times that of the control; and the amount of necrosis on the resistant variety (Strain 28949) ranged from 2.1 to 2.8 times that of the control. The results were statistically significant.

When leaves from mildew-free plants of the susceptible variety were excised, inoculated, and maintained on a sucrose solution to which various amounts of thiamin were added, there was no statistically significant effect on mildew development as compared with the control. There was a tendency, however, toward increase of mycelial development with the lower concentrations of thiamin. Thiamin solution applied to soil supporting mildew-free plants of the susceptible variety from which leaves were finally excised, inoculated, and maintained on a sucrose solution, significantly increased mildew growth at 0.01 p.p.m. concentration; but higher concentrations of the watering solution were without consistent significant effect.

Various concentrations of thiamin solution added to soil had no consistent significant effect on the green weight or height of cantaloupe plants growing therein.

U. S. HORTICULTURAL FIELD STATION,
BOX 150, LA JOLLA, CALIFORNIA.

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MOLDY CORE OF APPLES IN WISCONSIN

J. B. CARPENTER[1]

(Accepted for publication February 14, 1942)

Studies on moldy core of apples have been reported by several investigators. Harrison (4) reviewed most of the literature prior to 1935 and presented the results of comprehensive studies on Gravenstein apples in Nova Scotia. Goss and Doolittle (3), 1915, and MacInnes (5), 1917, reported studies on moldy core. In New Zealand, Brien (1) published recently on the fungi associated with moldy core. Tiller and Cooper (7) investigated the detection of moldy core by X-ray but found it commercially unfeasible. Woodhead (8) found no correlation between pruning practices and the incidence of this disorder. In Argentina moldy core has been studied by Marchionatto (6).

Moldy core occurs annually in susceptible varieties throughout Wisconsin, but it is of minor economic importance. The work reported herein was done at Gays Mills, Wisconsin, using chiefly Dudley and Delicious apples, locally the most susceptible varieties. Dudley, the more important variety in the Gays Mills area, is marketed as a green culinary apple without the complications that might arise from prolonged storage. The losses result from a lowering of the grade of the few fruits visibly affected at the time of picking. Moldy core in Delicious may prove objectionable in advanced cases, since the blackened core cavity is unsightly in a dessert apple. Fortunately, flesh rot is not commonly associated with this disorder in Wisconsin.

MATERIALS AND METHODS

The apples used were of typical conformation and free from complicating defects, such as internal injuries caused by insects or externally visible growth cracks. The samples were picked to represent both the typical exposures and the various size classes of fruit.

The Dudley and Delicious fruits used were usually immature. The samples of Dudley fruit were taken from shortly before harvest until well into the harvest period in 1939 and 1940. In 1941 the samples were picked within a 10-day period before harvest. The Delicious apples were sampled in the latter part of August, a few weeks before their harvest time. When measurements of fruit size were made, the maximum transverse diameter was determined.

To isolate the fungi associated with moldy core and to determine the prevalence of the disorder, a piece of endocarp or a seed was removed aseptically from each fruit and transferred to a Petri dish containing potato-dextrose agar. The plates were incubated for at least 1 week at room temperature. In fruit with no macroscopic symptoms a piece of endocarp was re-

[1] The writer wishes to thank Dr. G. W. Keitt for helpful criticism and advice during the course of this study.

moved near the distal end of the core, since the fungus enters there. In 1941, moldiness was macroscopically visible in only 62 per cent of the affected fruit. The fungi isolated were identified microscopically, although their gross cultural characters were usually definitive.

EXPERIMENTAL RESULTS AND DISCUSSION

The symptoms of moldy core observed are like those described by Brien (1) and Harrison (4). The endocarp walls and the seeds gradually become overrun and darkened by fungal growth, and in the advanced stages the core cavity is filled with a gray mycelial weft. Occasionally the flesh surrounding the core begins to rot as the fruit approaches maturity. The only reliable color change associated with this disorder in Dudley and Delicious is a precocious development of the yellow ground color, and this occurs only when the infection is at an advanced stage. The flavor of the fruit is unimpaired, unless flesh rot occurs.

Most of the affected fruits had no external symptoms at their respective harvest times. Slight moldiness is not an important defect in fruit for immediate consumption. The growers of the Gays Mills area report no complaints about the development of moldy core into a flesh rot in stored Delicious apples.

The disorder was about equally common in Dudley fruits of all sizes (Fig. 1). Observations indicate a like relationship for Delicious. Harrison (4) found among Gravenstein apples that moldy core was more common in the heavier fruit.

TABLE 1.—*Fungi associated with moldy core in several varieties of apple, Gays Mills, Wisconsin*

Variety of apple	Year	Isolations attempted	Fruits yielding			
			Alternaria sp.	*Fusarium* spp.	*Alternaria* sp. and *Fusarium* spp.	Other fungi
		No.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ben Davis	1939	36	27.8	0.0	2.8	2.8
	1940	62	14.5	0.0	0.0	3.2
Delicious	1939	34	79.4	8.8	5.8	2.9
	1940	105	48.5	2.9	8.6	7.6
	1941	150	65.3	7.3	9.3	2.7
Dudley	1939	56	76.8	1.8	10.7	1.8
	1940	301	78.1	0.9	1.9	2.6
	1941	403	71.7	3.7	9.9	8.4
Fameuse	1940	60	6.6	0.0	1.5	1.5
Golden Delicious	1940	29	10.3	0.0	0.0	0.0
McIntosh	1940	51	2.0	2.0	0.0	3.9
Northwestern Greening	1940	51	4.0	2.0	0.0	0.0
Wealthy	1940	51	11.6	0.0	0.0	4.0

It is well known that Delicious apples usually have a calycine sinus connecting the central core chamber with the calyx tube. In the present work this structure was found to exist in Dudley, though less commonly. In its

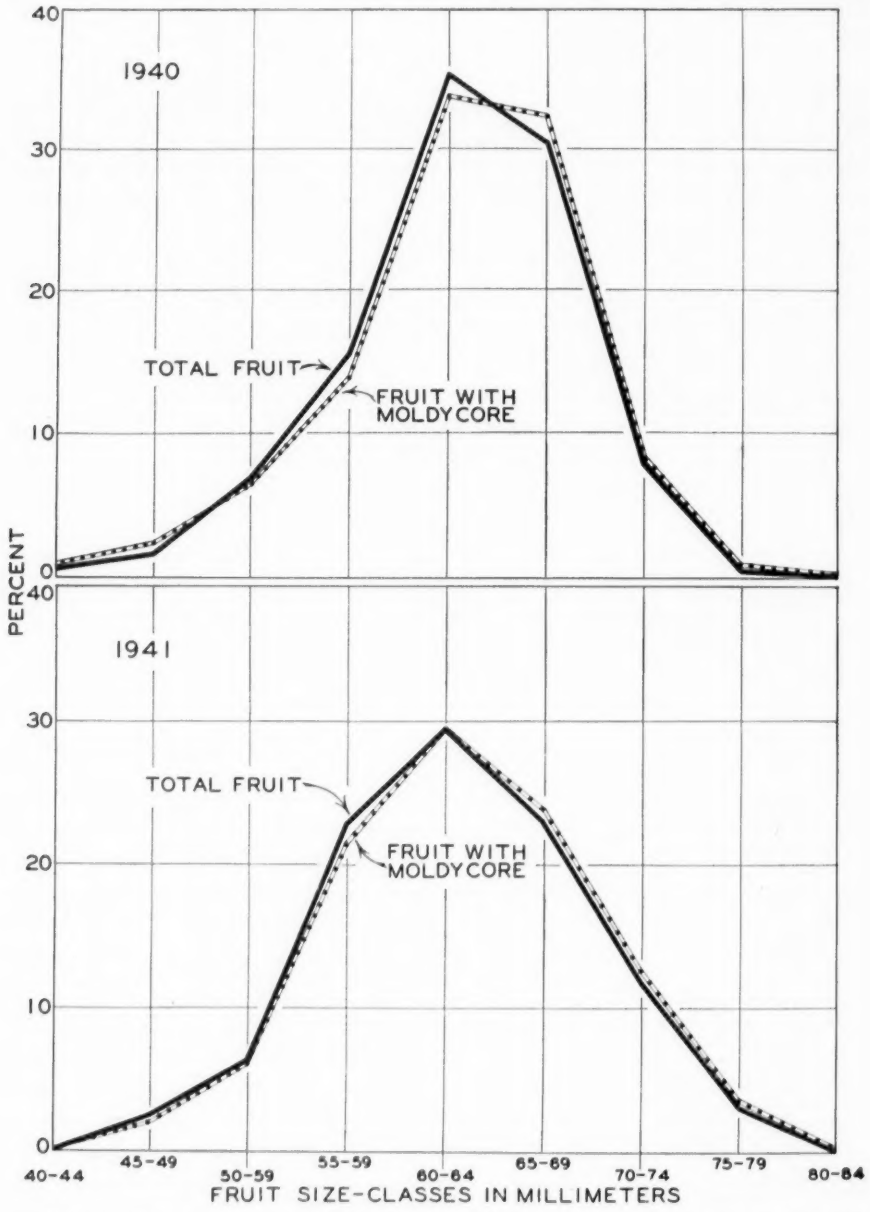

FIG. 1. The incidence of moldy core in relation to the size of Dudley apple fruits. In 1940, 222 of 275 fruits were infected and in 1941, 378 of 402.

absence in either variety, the distal end of the core cavity was found to be separated from the calyx tube by only a thin layer of tissue. Minute cracks

may occur in this layer, or the style bases may unite imperfectly, leaving a small opening into the core. In varieties not commonly affected with moldy core, there is no calycine sinus, and usually a few millimeters of apparently solid tissue lie immediately below the calyx tube, effectively barring the entrance of chance invaders.

The fungi associated with moldy core were predominantly of the genus *Alternaria* (Table 1). The isolates of this genus all fell within the limits of the *Alternaria tenuis* group (2). In each season, 1939, 1940, and 1941, the morphological characters of the many isolates were uniform enough to make them members of a single species. Spore size was as variable in monoconidial as in mass isolates. Inasmuch as the *Alternaria tenuis* group includes many named species of uncertain validity, the isolates of *Alternaria* considered in this study will be hereinafter designated as *Alternaria* sp.

Alternaria sp. was occasionally associated with a species of *Fusarium* or with certain other fungi. An isolate of *Fusarium* or of some undetermined genus was obtained alone from a few fruits.

An attempt was made to identify the organisms associated with the flesh rot that occasionally accompanied moldy core. Isolates of both *Alternaria* and *Fusarium* were obtained from the rotted flesh.

Species of *Alternaria*, *Coniothyrium*, *Fusarium*, and *Hormodendrum*, as well as bacteria or yeasts, were isolated in abundance from the floral remnants in the calyx basin. The isolates of *Alternaria* and *Fusarium* were morphologically identical with those from moldy cores. The presence of so many different fungi in the calyx basin, considered with the predominance of *Alternaria* sp. in moldy cores, suggests that the latter fungus may be peculiarly adapted for penetrating into the core and establishing itself (*cf.* 4).

SUMMARY

An account is given of the symptoms of moldy core in the Dudley and Delicious varieties of apple. This disorder occurs in a high proportion of the fruit of these varieties in Wisconsin, but it is of minor economic importance.

Moldy core was equally common in Dudley fruit of all sizes.

Undesirable morphological characters of the fruit predispose Dudley and Delicious apples to infection by the several fungi found in moldy cores.

A species of *Alternaria* of the *Alternaria tenuis* group was the predominant fungus. Species of other genera were found either alone or associated with *Alternaria* sp.

Inasmuch as several species of fungi were found growing in the calyx basin on dead floral remnants, it seems that *Alternaria* sp. may be peculiarly adapted for entering the core and establishing itself.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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A NEW STRAIN OF THE TOMATO LEAF-MOLD FUNGUS (CLADOSPORIUM FULVUM)

L. J. ALEXANDER[1]

(Accepted for publication February 12, 1942)

INTRODUCTION

In the development of the leaf-mold-resistant tomato variety, Globelle, Alexander (2) crossed individuals of the resistant currant tomato, *Lycopersicon pimpinellifolium* Mill. with individuals of the domestic species, *L. esculentum* Mill. varieties Globe and Marhio. During the second crop after the introduction of the variety Globelle, leaf-mold lesions were observed on this variety, and subsequently it was attacked where grown throughout northern Ohio. Observations indicated that a new strain of the fungus had appeared.

Guba (6) studied the resistance of the tomato to Cladosporium leaf mold and came to the conclusion that the existing confusion regarding the partial resistance of certain varieties of the domestic species was due to variability in the growing conditions rather than to the existence of different strains of the fungus. Langford (8), in a study of the parasitism of *Cladosporium fulvum* Cooke, identified 4 physiologic strains of the fungus. However, both Guba and Langford, and others (1, 2, 4 and 9), reported that *Lycopersicon pimpinellifolium* was highly resistant to or immune from all collections of the fungus tested. Also, in private correspondence, D. H. Wenholz of New South Wales reported *L. pimpinellifolium* highly resistant to *C. fulvum*.

Alexander (3) reported the occurrence of a new strain of the fungus capable of attacking Globelle, as well as the collection of *Lycopersicon pimpinellifolium* previously found resistant or immune. This paper is an elaboration of the earlier article and presents additional evidence to show that *Cladosporium fulvum* is not limited in its host range to varieties of the domestic species.

METHODS AND MATERIALS

In a previous article, the writer (1) described the methods of inoculation and disease recording. In the present investigation, the same methods were followed, except that diseased leaf materials, used as a source of spores for inoculation, was collected from different sources. Diseased leaves of Globelle were collected from the glasshouse where the disease first appeared on this variety. Diseased leaves from the susceptible variety Globe were collected from glasshouses in southern Ohio, where Globelle was not adapted and, consequently, had not been grown. In this paper, the spores collected from Globe will be referred to as the "Globe strain" and those collected from Globelle will be referred to as the "Globelle strain."

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

EXPERIMENTAL

In cross-inoculation tests, Globe and 3 other standard varieties were used as susceptibles. The resistant varieties were Red Currant, Globelle,

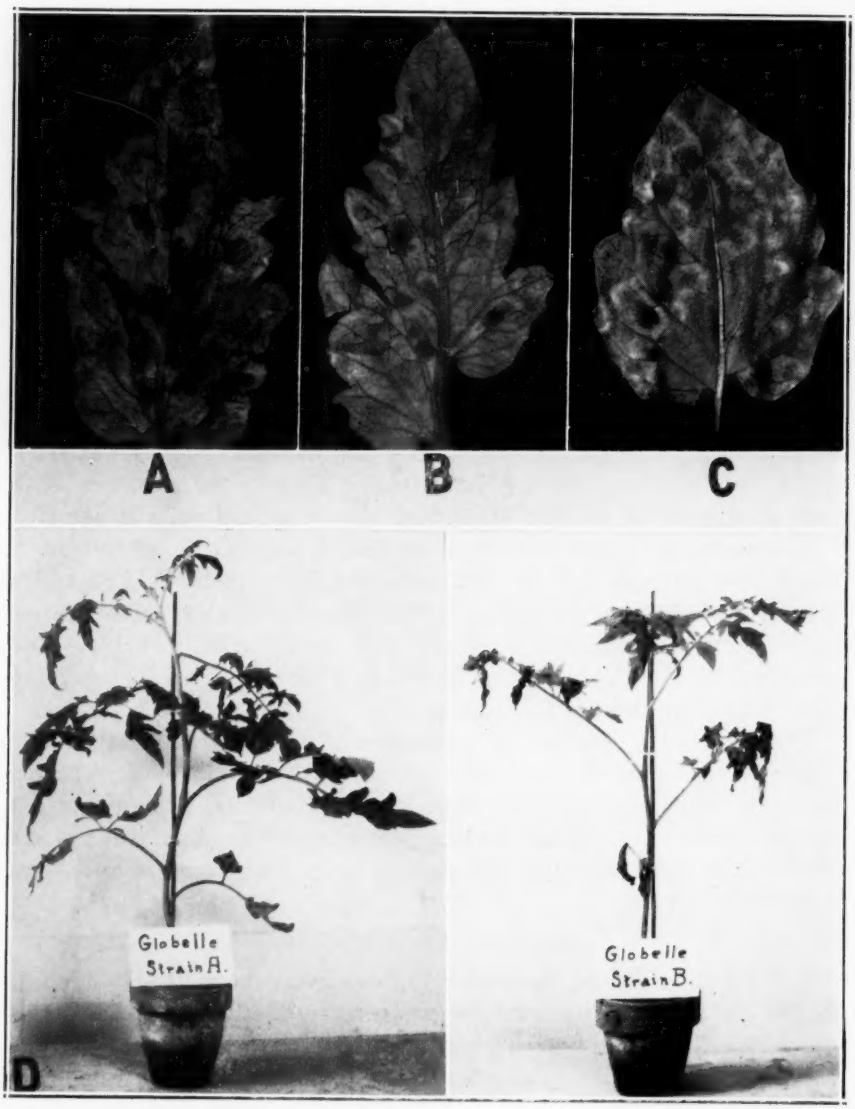

FIG. 1. Leaflets of Globe (A), Globelle (B), and Red Currant (C) naturally affected with leaf mold. D. Plants of the variety Globelle artificially infected with the Globe (left) and Globelle (right) strains of the leaf-mold fungus.

Bay State, and Veto-mold. The two last-named varieties were introduced as leaf mold-resistant by Guba (7) and Langford, respectively.

The results of 4 separate cross-inoculation experiments are combined and summarized in table 1.

All plants of the standard varieties were susceptible alike to both strains of the fungus. However, Red Currant and the three resistant varieties were highly resistant to or immune from the "Globe strain," but were susceptible to the "Globelle strain" of the fungus. This difference is illustrated in figure 1, D. In the course of additional work to improve Globelle, these findings with respect to Globe and Globelle have been confirmed. As a further test of the resistance of Red Currant, a few plants were grown along with Globelle in a commercial glasshouse. When the disease appeared on Globelle, it also appeared on Red Currant. A photograph of a naturally infected leaflet of Red Currant is shown in figure 1, C.

Since its introduction, Globelle has been grown in many glasshouses,

TABLE 1.—*Summation of four experiments to demonstrate the existence of two strains of Cladosporium fulvum*

Varieties tested	Globe strain		Globelle strain	
	No. plants resistant	No. plants susceptible	No. plants resistant	No. plants susceptible
Globe	0	61	0	81
Bonny Best	0	8	0	8
Potentate	0	20	0	20
Stirling Castle	0	27	0	26
Red Currant (*L. pimpinellifolium*)	30	0	0	30
Globelle	82	0	0	95
Bay State	16	0	0	15
Veto-mold	31	0	0	16

hence there has been an opportunity to make comparative observations on the severity of the disease on the varieties Globe and Globelle. These observations indicate that Globelle is injured less severely than Globe. Lesions develop more slowly on the one than on the other; consequently, it takes longer for leaves to be killed. This difference is illustrated in figure 1, A and B. The light-colored areas on the leaflet of Globelle are leaf-mold lesions that are developing slowly and have not yet fruited.

In a cursory microscopic examination of spores and fruiting structures of both strains of the fungus, no significant differences were noted. Measurements of the unicellular spores of the "Globelle strain" of the fungus averaged $3.89 \times 1.80\ \mu$; spores of the "Globe strain" averaged $3.84 \times 1.74\ \mu$. This slight difference is probably not significant.

DISCUSSION

The origin of the new strain of the fungus is uncertain. The organism is interesting because it was first observed in a commercial glasshouse where many parental lines of Globelle had been grown without showing any evidence of leaf mold. This observation in connection with those of other investigators (4, 6, 8, and 9) who had not observed any evidence of the existence of strains of the fungus capable of attacking the currant tomato indicates that the new strain of the fungus was not previously present.

These observations suggest the possibility that the new strain arose by mutation.

The first observed lesions of the new strain of the fungus were few in number and not typical of leaf mold, but a microscopic examination revealed the presence of typical *Cladosporium* spores and conidiophores. The succeeding crop of Globelle became generally infected. These lesions were typical of leaf mold and some defoliation occurred. Subsequent to the first appearance of the new strain of the fungus it soon appeared in glasshouses throughout northern Ohio and, also, reports of its existence have come to the author either by letter or by conversation with plant pathologists or growers from the greater part of the northeastern United States and once from Texas.

If the new strain of the fungus arose, as suspected, by mutation, it seems possible that seed transmission could account for its rapid dissemination. Opportunities for seed infection were present because much of the early seed of "Globelle" was sold by the grower in whose glasshouse the new strain first appeared. This seed was saved from ripe to over-ripe fruits, picked from vines infected with the Globelle strain. The pulp was extracted and allowed to ferment in the packing shed and finally put to dry on screens in a small plant house. In this connection, it might also be mentioned that Gardner (5) demonstrated that seed transmission can occur when fruits are severely damaged by the leaf-mold fungus.

SUMMARY

The germplasm of the imperfect fungus, *Cladosporium fulvum*, is shown to be sufficiently plastic to permit the evolution of new physiological strains.

The fungus, *C. fulvum*, is not limited in its parasitism to the one host species, *Lycopersicon esculentum*.

The variety Globelle, introduced as resistant to leaf mold, proved susceptible to the new strain of the fungus but less susceptible than Globe.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
OHIO AGRICULTURAL EXPERIMENT STATION,
WOOSTER, OHIO.

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DEFOLIATION OF AMERICAN HOLLY CUTTINGS BY RHIZOCTONIA

J. S. Cooley

(Accepted for publication February 24, 1942)

INTRODUCTION

A disease of American holly (*Ilex opaca* Ait.) cuttings was observed in 1936 in propagation experiments of Neil W. Stuart and Paul C. Marth[1] at this station. In the following year these cutting beds were emptied and refilled with clean sand, but the disease again caused heavy loss. It was noted that the disease first appeared near a wooden partition and rapidly spread to the rest of the cutting bed.

Holly cuttings rooted best when set deep, with the petioles and often part of the leaf blades covered with sand. This deep setting also favored development of the pathogen in question, a species of Rhizoctonia. Because holly cuttings require a month or more to root and the favorable period for propagation may be passed before the disease becomes manifest, it is difficult to replace losses from this disease by starting other cuttings later.

SYMPTOMS

The first symptom of the disease is a cobweb-like effect (Figs. 1 and 2) resulting from adherence of fungous filaments and grains of sand to the under side of leaves that touch the sand. Defoliation may begin in 2 or 3 weeks after the cuttings are set (Figs. 1 and 2). Not long after defoliation black sclerotia may develop on the dead leaves lying on the sand. Sclerotia do not always form on leaves in the cutting bed, but they usually form very readily on leaves of cuttings placed in a glass moist chamber. Another symptom that is sometimes, but not always, present is a zonate leaf spot. It developed on naturally infected cuttings and on cuttings in beds inoculated with a pure culture of the fungus (Fig. 1), but not in uninoculated beds. Isolations from such zonate spots yielded a Rhizoctonia that produced characteristic defoliation symptoms when used in inoculations. This evidence justifies the conclusion that under certain conditions this pathogen which is identical with the species of Rhizoctonia used in other inoculation experiments, may produce a zonate leaf spot. Stahel[2] reported a species of *Corticium* causing a zonate or areolate leaf spot on citrus in Surinam.

PATHOGENICITY

A species of Rhizoctonia consistently isolated from diseased cuttings has proved to be the cause of this disease. On December 11, 1937, sterilized sand was placed in glass moist chambers and mixed with inoculated oats; one dish

[1] Stuart, Neil W., and Paul C. Marth. Composition and rooting of American holly cuttings as affected by treatment with indole butyric acid. Proc. Amer. Soc. Hort. Sci. **35**: 839–844 (1937). 1938.

[2] Stahel, Gerold. *Corticium areolatum*, the cause of the areolate leaf spot of citrus. Phytopath. **30**: 119–130. 1940.

was left uninoculated to serve as a check. Cuttings were then set in these moist chambers as in a cutting bed. Ten days after setting, the cuttings in the inoculated sand had dropped part of their leaves and nearly all were about to drop. Fungus filaments with enmeshed grains of sand adhering to the under side of the leaves presented the cobweb effect characteristic of the disease in a cutting bed (Fig. 1). Blackening of petioles and of parts of

FIG. 1. Diseased holly cutting from an inoculated cutting bed, showing the advance of the disease up the petioles, the adherence of sand to the affected parts, and the zonate leaf spot (indicated by arrow).

leaf blades was also apparent. The symptoms experimentally produced on holly cuttings in a moist chamber by a pure culture of the Rhizoctonia were therefore similar to those in naturally infected holly cuttings from which the Rhizoctonia was isolated. The reisolated fungus was identical with the inoculant. Cuttings in the moist chamber containing uninoculated sand were alive and showed no evidence of the disease at the conclusion of the test. Later experiments with cuttings set in inoculated and in disease-free beds (Fig. 2) confirmed this evidence obtained from moist chambers.

Cultures of this fungus were determined to be *Rhizoctonia solani* Kühn,

FIG. 2. Two healthy holly cuttings from uninoculated cutting bed (left). Two diseased cuttings from inoculated bed; a zonate leaf spot is indicated by the arrow (right).

by William Davis and by Ernest Wright of the Division of Forest Pathology, U. S. Bureau of Plant Industry. Comparison of this strain with a species of *Rhizoctonia* isolated from potato showed only minor differences in color and character of growth. This fungus proved to have a very narrow temperature range for good growth in Petri plates of potato-dextrose agar, as recorded in Table 1. Growth was extensive at temperatures near 25° C., but

TABLE 1.—*Effect of temperature on growth of Rhizoctonia from holly on potato-dextrose agar*

Temperature	Diameter of hyphal mat after 48 hours	Character of hyphal mat
° *C.*	*Mm.*	
5	0	.....
10	0	.....
15	16.8	Very thin
20	41.5	Thin
25	73.5	Very dense
30	55.6	Thin
35	0	.....
40	0	.....

very restricted above 30° or below 15° in the 48-hour period. At 20° C. there was considerable elongation of hyphae but the hyphal mat was very thin, and at 15° it was decidedly subnormal in appearance. In the presence of sufficient moisture the development of this disease may be dependent upon suitable temperature. Holly cuttings rooted favorably at a temperature near the optimum for growth of this fungus, but the effect of temperature on rooting was not a subject of experimentation.

Cuttings of a number of plants usually propagated by cuttings were set

in a bed infested with the holly Rhizoctonia and also in an uninoculated bed. This test included begonia (*Begonia* sp., Lorraine type), buddleia (*Buddleia davidi* Frauch), carnation (*Dianthus caryophyllus* L.), chrysanthemum (*Chrysanthemum* sp.), fuchsia (*Fuchsia* sp.), geranium (*Pelargonium hortorum* Hort.), heliotrope (*Heliotropium* sp.), lantana (*Lantana camara* L.), peristrophe (*Peristrophe angustifolia* Nees.), and snapdragon (*Antirrhinum majus* L.). All the plants tested showed some degree of infection and gave much poorer rooting in the inoculated than in the non-inoculated bed. In the inoculated bed cuttings of highly susceptible plants, such as begonia, died in a short time while more resistant ones, such as the chrysanthemum, showed moderate injury with survival of some of the cuttings. These results indicate that the holly Rhizoctonia may kill the cuttings or adversely affect the rooting of a wide variety of plants.

SOURCE OF INFECTION

When cuttings from different holly trees and from different positions on such trees, including limbs touching the ground, were set in sterilized sand in moist chambers no disease developed. Further evidence that holly cuttings are not the usual source of infection was supplied by other experiments reported in this paper in which holly cuttings taken from the open and set in a clean cutting bed remained disease-free throughout the rooting period. Although this evidence does not preclude the possibility that holly cuttings may sometimes carry the fungus, it is probable that infection usually originates from contaminated greenhouse material.

CONTROL EXPERIMENTS

In testing chemical treatments the cutting bed was divided into two comparable parts; one was inoculated with the pathogen, and the other was kept disease-free, as a check on the effect of the chemical or other treatments on disease-free cuttings. The fungus, cultured on either steamed oats or Tochinai's[3] solution, was allowed to grow in the inoculated portion of the cutting bed for 2 to 3 days before the chemicals were applied either in solution or in suspension. The propagating case was then kept closed for 12 or more days before the cuttings were set, and again after they were set. The bed was supplied with electrical bottom heat thermostatically controlled at 24° to 27° C. When most of the cuttings were well rooted the bed was cleared and the results recorded. Such experiments were carried on in four successive years.

None of the chemicals tested[4] afforded control of the disease under the conditions of the tests; and all treatments reduced the proportion of rooted cuttings in the uninoculated bed. Applications of disinfectants to cuttings after they were set resulted in chemical injury without control of the disease.

An important result of these experiments was the absence of diseased

[3] Tochinai, Y. Comparative studies on physiology of *Fusarium lini* and *Colletotrichum lini*. Jour. College of Agr. Hok. Kardo Imp. Univ. **14**: 171–236. 1936.

[4] Acetic, sulphuric, hydrochloric, a-naphthyl acetic, and phosphoric acids, sulphur, ferrous sulphate, potassium permanganate, cuprous oxide, aluminum sulphate, ammonium sulphate, calcium superphosphate, Bordeaux mixture, mercuric chloride, at various concentrations.

plants in the noninoculated bed, which was separated from the heavily infected bed by a 6-inch board only. In preparation for the experiment, the bed had been cleaned of sand, disinfected with 1:50 formaldehyde solution, and refilled with clean fresh sand. Care was exercised to prevent the carrying of the fungus from the diseased to the noninoculated bed.

Throughout 4 years shallow setting of cuttings also was tested as an accessory control measure. Cuttings were set in a contaminated and also in an uninoculated cutting bed; half of the cuttings in each bed were set shallow so that no leaves were covered with sand, the rest were set deep, so that the petioles and part of the leaf blades were covered with sand. Usually, after about a week, the deep-set cuttings in the contaminated bed showed fungus growth and some leaf shedding, while the shallow-set cuttings showed no evidence of leaf shedding. After 2 weeks the deep-set cuttings showed considerable rhizoctonia infection and defoliation, while the shallow ones showed infection and defoliation only where they were set too close and the leaves touched each other or the sand. In the noninoculated beds both deep-set and shallow-set cuttings consistently remained healthy. Results do not justify recommending shallow setting as a sole perventive measure.

Since *Rhizoctonia solani* does not readily sporulate, dissemination by spores is not common. The fungus, however, does produce an abundance of hyphal growth, which is readily disseminated as mycelial strands and fragments that adhere to various objects. Although the disease is thus readily spread, it has been possible to maintain an experimental disease-free bed adjacent to a heavily diseased one. These experiments show that sanitation affords practical control. If the disease has been present in a bed, the sand should be removed and the bench washed with a disinfectant, such as 2 per cent formaldehyde or 2 per cent copper sulphate solution. The bed should be refilled with fresh clean sand or sterilized sand, and every effort should be made to prevent recontamination. Since *Rhizoctonia* may live on plant debris in the soil, it seems best to avoid taking cuttings from holly branches that touch the ground.

SUMMARY

A leaf-drop disease of American holly cuttings, caused by *Rhizoctonia solani*, is described. The pathogenicity of the fungus was demonstrated by the usual means. The fungus in culture showed a restricted temperature range with the optimum between 25° and 30° C. No positive evidence was obtained that the disease is harbored on holly plants from which cuttings were made. When heavily contaminated cutting beds were treated with a number of disinfectants and cuttings were set after a lapse of 12 or more days none of the disinfectants gave satisfactory control. However, thorough sanitary measures gave effective control. These included renewal of the cutting bed with fresh clean sand after washing the bench with a disinfectant, and the taking of precautions against recontamination.

U. S. BUREAU PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

RELATIVE CONCENTRATIONS OF TWO STRAINS OF CURLY-TOP VIRUS IN TISSUES OF SUSCEPTIBLE AND RESISTANT BEANS

C. F. LACKEY

(Accepted for publication February 17, 1942)

While investigating the relationship of curly-top virus to root tips of sugar beets it was sometimes difficult to obtain sufficiently large root tips from diseased beets. This difficulty directed attention to beans (*Phaseolus vulgaris* L.), which, in the early stages of curly top, produce somewhat larger root tips. Therefore, in some of this work bean root tips were studied along with root tips of sugar beets.

This paper is an account of experimental results on factors affecting virus concentration in beans. Two strains of curly-top virus served as inoculum for 2 varieties of beans differing in resistance to curly top.

Curly-top virus strains 1 and 4 were chosen from those described by Giddings.[1] The bean varieties were Great Northern U.I. 81, a selection made by Pierce[2] and reported somewhat resistant to curly top by Murphy,[3] and Bountiful, an extremely susceptible commercial variety. Under greenhouse conditions and when inoculated on young leaves, U.I. 81 continues to grow, although obviously affected by virus strain 1. With Bountiful, however, Giddings[1] showed that infection by virus strain 1 caused a loss of leaves, complete cessation of growth, and early death.

Plants of each variety were inoculated with curly-top virus strain 1 and later the root tips and shoot tips were tested for virus concentration, the former about 1 week after inoculation. In each experiment 20 root tips (5 mm. length) of each variety were cut and macerated separately in a 5 per cent sucrose solution. Non-viruliferous beet leaf hoppers were fed on these preparations and then caged singly on susceptible sugar beet seedlings. The percentages of infection in the parallel series of test plants afforded a comparison of the virus concentrations in the 2 bean varieties. Shoot tips were tested by expressing the juice and feeding it to non-viruliferous leaf hoppers.

Table 1 shows the results of parallel experiments with curly-top strain 1 on the 2 bean varieties. Since the number of test plants varied among the experiments, infections are shown as percentages. These results show that in 19 out of 20 tests more of the beets became infected when inoculated with virus from the susceptible bean variety than when inoculated with virus from the resistant variety. In the 13th test, percentages of infection were equal. Obviously, the virus was regularly more concentrated in the root

[1] Giddings, N. J. Studies of selected strains of curly top virus. Jour. Agr. Res. [U.S.] **56**: 882–894. 1938.

[2] Pierce, W. H. Resistance to common bean mosaic in the Great Northern field bean. Jour. Agr. Res. [U.S.] **49**: 183–188. 1934.

[3] Murphy, Donald M. A Great Northern bean resistant to curly top and common bean mosaic viruses. Phytopath. **30**: 779–784. 1940.

TABLE 1.—*Infection percentages on test beet plants, indicating comparative concentrations of curly-top virus strain 1 in tips of roots and shoots of (a) susceptible bean variety (Bountiful) and (b) resistant variety (U.I. 81)*

Experiment	Virus source			
	Susceptible variety		Resistant variety	
	Number of sugar beets inoculated	Percentage infected	Number of sugar beets inoculated	Percentage infected
Extract from root tips used[a]				
1	24	43.5	24	4.2
2	24	50.0	24	0.0
3	24	20.8	24	0.0
4	24	50.0	24	0.0
5	24	25.0	24	0.0
6	13	38.6	15	6.7
7	22	63.6	24	20.8
8	20	55.0	20	5.0
9	24	50.0	24	4.2
10	20	25.0	15	6.3
11	20	35.0	20	5.0
12	20	65.0	20	0.0
13	20	5.0	20	5.0
14	20	70.0	24	4.2
15	20	35.0	20	5.0
Expressed juice from shoot tips used				
16	20	50.0	20	0.0
17	20	10.0	20	0.0
18	20	30.0	20	10.0
19	16	43.8	20	10.0
20	20	25.0	20	0.0

[a] 20 root tips (5 mm. long) of each variety were macerated in 5 per cent sucrose solution.

tips of the susceptible variety. Limited evidence indicates the same is true for shoot tips.

In experiments by Giddings[4] and in some of the tests included in table 1

TABLE 2.—*Infection percentages on test beet plants, indicating comparative concentrations of virus in root tips of Bountiful beans infected with (a) curly-top virus strain 1 and with (b) curly-top virus strain 4*

Experiment	Curly-top virus strain 1		Curly-top virus strain 4	
	Number of sugar beets inoculated	Percentage infected	Number of sugar beets inoculated	Percentage infected
1	24	50.0	24	12.5
2	32	34.4	14	14.3
3	24	45.8	24	0.0
4	24	37.5	24	16.7
5	22	63.7	22	27.3
6	20	60.0	24	0.0
7	15	26.7	12	25.0
8	20	70.0	20	30.0
9	20	35.0	20	10.0

[4] Giddings, N. J. Unpublished data.

it was observed that a small amount of the virus was usually obtained from U.I. 81 plants soon after symptoms were detectable, but very rarely in later periods of growth of the infected plants. Apparently, shortly after infection occurs some factor in this resistant variety inactivates the virus or inhibits its further multiplication.

Further studies were conducted in which virus strain 1 and the less virulent strain 4 were used to inoculate the susceptible variety Bountiful. Tests of virus concentration in the root tips were conducted as before. These results reveal that in all cases infection percentages were greater when inoculations were made with virus from beans infected with strain 1 (Table 2). The differences were large in 8 of the 9 tests made. Evidently the concentration of the virulent strain was consistently higher than that of the less virulent strain.

These tests demonstrate that in the resistant bean, U.I. 81, virus strain 1 is promptly inactivated or multiplication is quickly inhibited. In highly resistant sugar beets, on the contrary, Giddings[1] has found strain 1 to persist. The tests also show that in the root tips of the susceptible bean, Bountiful, the virulent strain 1 promptly reaches a higher concentration than does the less virulent strain 4. This behavior is in contrast to that found in root tips of susceptible beet varieties[5] in which strain 4 reaches a slightly higher concentration than does strain 1.

DIVISION OF SUGAR PLANT INVESTIGATIONS,
U. S. DEPARTMENT OF AGRICULTURE,
RIVERSIDE, CALIFORNIA.

[5] Lackey, C. F. Unpublished data.

PHYTOPATHOLOGICAL NOTE

Susceptibility of Solanum species to Fusarium solani var. *eumartii.*—The lack of information concerning the occurrence of fusarium wilt in wild tuber-bearing species of *Solanum,* and the possibility of discovering resistant parent material for breeding purposes, led the writers to test a number of species kindly supplied by Donald Reddick. The tests were made by growing the seedlings in inoculated, sterilized and unsterilized soil, as described by the writers.[1]

Typical foliage and tuber symptoms of the disease were produced in the seedlings, and the pathogen was recovered in pure culture. On the basis of these tests the following species proved susceptible to the disease: *Solanum acaule, S. demissum* (Reddick 178, 418, and 519), *S. chacoense, S. commersonii* ("Blanca" and "Colorado," from Las Delicias, Argentina), *S.* sp. from Chile, P. I. 129381, *S. antiporiczii.*—R. W. Goss and J. H. Jensen, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

[1] Jensen, J. H. and R. W. Goss. Infection of first-year potato seedlings with *Fusarium soloni* var. *eumartii.* Amer. Potato Jour. **18**: 239–242. 1941.

BOOK REVIEWS

HAYES, HERBERT KENDALL, and FORREST RHINEHART IMMER. *Methods of Plant Breeding.* (1st. ed. 432 pp., 37 figs. McGraw-Hill Book Co., Inc., (New York and London). 1942. $4.00.

A textbook designed primarily for the use of students and workers interested in field-crop improvement through plant breeding. Agronomists, phytopathologists, economic botanists, and other allied professional groups also should find this text especially valuable as a reference. The senior author, who has spent his entire career specializing in the teaching and practice of plant breeding, is unusually well qualified to prepare a work of this kind. Hence, many valuable data are presented and numerous outstanding examples of the best methods of breeding crop plants are discussed. The junior author's contribution to the book on methods of field-plot technique, experimental design, and statistical analysis is equally outstanding, presenting timely and up-to-date information on these subjects. Although still a young man, he has attained an eminent position among authorities in his chosen field of research.

In the preface, the authors have expressed concisely, yet rather completely, in simple language the general nature and use of the material presented in the text. "The subject matter presented . . . has been used in both undergraduate and graduate courses. . . . The undergraduate course is taught only to junior and senior students. The graduate courses are given for the purpose of teaching standardized methods of breeding for particular categories of breeding problems and to present the current viewpoint when the most desirable method of breeding is not so well known. This is with the belief that each of the various methods of hybridization, including the pedigree method of selecting during the segregating generations, the bulk method with self-pollinated plants, the backcross method, and convergent improvement, has certain advantages and disadvantages that make it desirable under some conditions and less desirable for other breeding problems."

"Methods of field plot technic, experimental design, and statistical analysis with particular reference to plant breeding problems have been discussed, including some of the newer methods. The necessary statistical tables have been included with the permission of the original publishers."

The book consists of 22 chapters, and is illustrated with 37 figures comprising half-tones, tables, and graphs. The general topics for each chapter are: The role of plant breeding; the genetic and cytogenetic basis of plant breeding; mode of reproduction in relation to breeding methods; techniques in selfing and crossing; the pure-line method of breeding naturally self-pollinated plants; hybridization as a method of improving self-pollinated plants; the backcross method; breeding for disease and insect resistance; inheritance in wheat, oats, barley, and flax; methods of selection for special characters; development of methods of corn breeding; inheritance in maize; controlled pollination methods of breeding cross-pollinated plants; seed production; some commonly used measures of type and variability; field-plot technic; randomized blocks, Latin squares, and χ^2 tests; correlation and regression in relation to plant breeding; and multiple experiments, including methods of testing a large number of varieties and the analysis of data expressed as percentages. These chapter titles briefly set forth the contents of the book, which might be considered sufficient for a review, even without further comment.

The authors have deemed it unwise to attempt a complete review of the present status of the genetics of many crop plants, because such a review soon would become obsolete, so rapid is the advancement in the field of genetics, or more particularly in the field of cytogenetics. Brief reviews of the mode of inheritance of important characters of leading crop plants, such as corn, a typical cross-pollinated plant, and the small grains, such as wheat, oats, and barley, typical close-pollinated plants, have been included to illustrate to the reader and student the value of a knowledge of inheritance as an aid to the planning and development of breeding programs. The present status of corn breeding, the leading cross-pollinated economic crop plant, is reviewed in considerable detail, since many of the studies made with corn and the results obtained therefrom are basic to a complete understanding of principles of breeding, particularly for other cross-pollinated plants.

The authors have emphasized, and rightly so, the newer developments in plant-breeding methods that have contributed much to crop improvement. These include polyploids in relation to plant breeding, colchicine as a polyploidizing agent, linkage groups in barley and corn, convergent improvement in corn, the artificial production of epiphytotics of the rusts and smuts of the small grains, wheat-meal fermentation tests, etc. Special emphasis also is placed on the value of backcrossing and backcrossing-breeding programs to obtain more quickly certain goals in plant breeding.

The chapter on seed production presents valuable information on the first increase of

seed of a new variety, seed certification and registration, crop improvement associations, etc.

In the preparation of this new book, it is evident that the former well-known and widely used text "Breeding Crop Plants," by H. K. Hayes and R. J. Garber has been used freely. It, therefore, may be stated in all fairness to the authors that "Methods of Plant Breeding" is "Breeding Crop Plants" revised, brought up-to-date with the addition of much new knowledge, particularly of cytogenetics, methods of field-plot technique, experimental designs, and statistical analysis. In short, it is a much more complete book than its predecessor, and undoubtedly will receive wide recognition.

The book is well organized, with the possible exception of the chapter on seed production, which may belong more properly to a text on crop production. It is well written and quite free from errors of diction, spelling, etc., and is very readable. In general, this is the most concise, yet complete, text on the subject which has appeared to date. As a consequence, "Methods of Plant Breeding" should meet with almost universal favor among all classes of students and research workers who are interested in crop improvement through plant breeding.

The book is adequately documented with a list of 401 literature citations. These include the more important contributions by workers to plant genetics, plant breeding, and other closely related subjects, and will be of great value to the student for reference. A glossary of genetic, plant-breeding, and taxonomic terms follows the section on literature citations. There is appended a group of tables presenting values and constants employed in statistical analysis. These necessary tables contribute much toward a "rounding out" of the text. They also add much to its usefulness.

If the book is deficient in any respect, it is in the number of illustrations. More illustrations of an appropriate nature would have materially improved the text; especially at this time when pictures and drawings are being used more and more to present cardinal facts in all fields of endeavor. Modern textbooks also call for some colored pictures for illustrating certain pertinent subjects, and add much to their appearance and value.

Unfortunately, a few of the halftones used in the book are quite poor and should be replaced by better ones in the first revision by the authors.—T. R. STANTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

REED, HOWARD S. *A Short History of the Plant Sciences.* The Chronica Botanica Company (Waltham, Massachusetts). 1942. 320 pp. $5.00.

This book is designed for the average graduate student in our universities, rather than for the specialist in science or in history. Recognizing the limitations of one individual in treating a subject so broad as the history of the plant sciences, the author has chosen to deal chiefly with subjects with which he felt more familiar, referring the reader to recent works in which other writers have treated certain important fields (systematic botany, phylogeny, paleobotany, genetics, plant breeding, evolution, and recent work on growth, tropisms, and hormones).

The book contains 320 numbered pages and 37 figures. The chapters and the pages devoted to each are as follows: Introduction, 6; gardeners and herbalists of antiquity, 23; the nascent period, 15; the retrogressive period, 12; the renascent period, 24; the seventeenth century, 14; the eighteenth century, 18; gardens and other things, 13; plant geography in the nineteenth century, 9; morphology, 19; cytology, 22; the water economy of plants, 21; the fixation of carbon by plants, 18; the assimilation of nitrogen, 12; the fixation and metabolism of nitrogen, 14; plant nutrition, 13; mineral constituents in metabolism, 12; mycology, 19; plant pathology, 24; significant names in the history of botanical science, 1.

The author observes that perhaps he has spent too much time on early developments. The reviewer regards the eight chapters that trace developments from the dawn of history through the eighteenth century as one of the most valuable parts of the book. Here a very scattered and difficultly accessible literature is given scholarly and well-documented treatment.

A short history of the plant sciences encounters heavier going in the chapters in which the several fields are treated. Out of a great mass of material that has not yet received adequate historical interpretation and evaluation by workers in the fields concerned, contributions are selected and interpreted. On the whole one marvels that a single individual, even with the benefit of counsel from colleagues in several fields, should have covered so much so well. Nevertheless, the reviewer finds many places, especially in the fields with which he is more familiar, in which he would prefer a different selection of material or a different interpretation. In the chapter on mycology, for example, Micheli's epoch-making work receives four lines, without mention of its nature or interpretation of its significance. Yet, Fontana, whose contribution was minor in comparison, is given more than a page. No mention is made of Buller's valuable account of Micheli's work in its

historical setting. What was more fundamental to the early development of mycology and the mycological basis for pathology than Micheli's experimental demonstration that fungi reproduce by means of spores and are therefore autonomous organisms, rather than evanescent products of fanciful or superstitious origin?

A difficulty inherent in the plan of the work is encountered in the attempt to trace the development of major lines of research to their current position. It is not surprising that in covering so broad a field and including recent work some minor contributions receive consideration, while others of much more significance do not, and that in some cases the position of the problem has been drasticallly changed by recent work not treated.

An error that should be corrected is the assignment to the career of Winogradsky in the list of 50 significant names in the history of botany the closing date of 1934. Colleagues advise the reviewer that Winogradsky was very much alive in 1938 and, insofar as they are informed, may still be.

The book should very usefully serve the purpose for which its author designed it, a guide to reading in the history of the plant sciences. As such it should be very welcome, not only to the graduate student, but to all who are seriously interested in its field. It should not be mistaken for what it was not intended, a definitive and authoritative history of this broad field. This can be written, if ever, only after developments in the many branches of plant science have been adequately interpreted and evaluated by those intimately familiar with the fields concerned. It is to be hoped that Professor Reed's stimulating work will encourage those who cultivate the several fields of plant science to put their historical houses in order, so that students may be able to see the development of their science in its true perspective and the task may be lightened for future writers who have the courage, energy, and talent to attempt to treat the history of broader fields.—G. W. KEITT, University of Wisconsin, Madison, Wisconsin.

THE WAR EMERGENCY COMMITTEE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

At the Dallas Meeting of the American Phytopathological Society, after a panel discussion lasting the entire afternoon, action was taken authorizing the appointment of a War Emergency Committee of the Society, the personnel of which was published in the September, 1942, issue of PHYTOPATHOLOGY.

The general objectives formulated by the committee are as follows:

1. Provide for more adaquate plant-disease quarantines, foreign and domestic, to guard against introduction and distribution of new and destructive disease organisms.
2. Intensify plant-disease surveys to detect as soon as possible new plant-disease introductions and show where control efforts should be concentrated.
3. Summarize and codify known control measures, make them easily comprehensible and available to extension men and growers, and encourage more adequate extension work in plant pathology.
4. Attempt to get necessary priorities on chemicals and machinery used in controlling diseases.
5. Concentrate effort on necessary experimentation and research designed to improve the effectiveness and economy of plant-disease control measures, by cultural practices, chemical treatments, and resistant varieties.
6. Summarize, and make available information regarding preservation of food and other products in storage and transit, and provide for necessary studies to meet new situations.
7. Scrutinize present basic and long-time research projects with a view to procuring support for those designed to yield facts and principles on which important procedures are based and those that could not be interrupted without serious loss of materials, accumulated results, and experience.
8. Maintain adequate personnel.

It is desirable and necessary to cooperate to the utmost with the United States Department of Agriculture, the State Colleges of Agriculture and Agricultural Experiment Stations, the National Research Council, industrial organizations, and all other agencies that may be useful in the attainment of the general objectives.

All of the subcommittees are functioning and have agreed to issue brief monthly reports and occasional summaries of pertinent information.

At the Summer Meetings of The American Phytopathological Society, at Toledo, Ohio, June 25 and 26, 1942, considerable time was devoted to a discussion of the work of the War Emergency Committee as a whole, and to reports of the subcommittees. Following is a brief summary of the viewpoints expressed and the actions taken:

It is being recognized increasingly that "Food will win the war and write the peace" as expressed by Secretary of Agriculture, Claude R. Wickard. It is evident that there are now very important problems in protecting crop plants against diseases; that the problems become more acute because of shifts in crop production required by the exigencies of the war, and because of the increased importation of propagative parts of plants from other countries and wider distribution within the United States.

To aid agriculture in its immediate war job, research plant pathologists are crystallizing accumulated research results into simple instructions on how best to check losses from serious plant diseases of important war-time crops, or Victory Gardens. They also are directly assisting extension workers and others in planning effective disease-control programs after studying local conditions and adapting scientific findings to local needs and resources. This involves a type of engineering study, or clinical investigation, essential to successful application, on the farm, of knowledge coming from basic research. Special short-time emergency studies and pilot tests, indispensable to efficient war production, were recognized as suitable for support from appropriate federal-grant funds. The great value of voluntary teamwork shown by the Society's group research activities argues in favor of handling many war-time regional and national plant-disease problems through similar cooperative arrangements.

The following fields of research were cited as among those important to the Nation's war effort: A better organized Nation-wide plant disease survey service, fostered by the survey subcommittee, for effective direction of crop protection programs; work on new and improved fungicides and crop-protection methods led by the fungicide subcommittee; development of disease-resistant crop varieties with coordinated local tests under special subcommittees; research led by the seed-certification and seed-treatment committees on problems basic to certification or treatment of seed and planting stocks to reduce losses from seed- and plant-borne diseases; research on rotations, chemical treatment, and cultural management of soils to reduce losses from soil-borne diseases; coordinated research on virus diseases of plants with entomologists helping on insect carriers; prompt inves-

tigation of newly discovered, potentially destructive plant diseases; research on diseases of new crops being grown to meet war-time shortages of oil, fibers, drugs, spices, etc.; more general study of soybean diseases; and work on effective home-made dusting, spraying and treating equipment where commercial equipment is unavailable.

The Society was urged to setup as a war measure a plan for cooperative preparation of annual summaries of progress in various fields of plant-disease research with the help of the subject-matter subcommittees under the Society's War Emergency Committee.

The subsequent general discussion brought out the fact that the Society's national and regional war emergency organization was well adapted for prompt exchange of research information. The necessity for adequate, coordinated plant-disease survey work was repeatedly emphasized. Helpfulness of specialists in performing identification for colleagues was commended. The *Plant Disease Reporter* was declared useful in facilitating such collaboration and for prompt dissemination of important new findings. Voluntary cooperation for adequate attack on many plant-disease problems was stressed. The tremendous national importance of plant-disease eradication and control programs was said to demand their maintenance at highest efficiency during the emergency.

Shortage of plant pathologists was declared to call for courage to discontinue work on diseases of plants not vital to the war effort to give more time for effective application of available research information. It was stated that growers of ornamentals would still demand assistance on their problems. Discontinuing fundamental studies, regardless of the plants involved, was held to be dangerous from the standpoint of the public interest.

It was generally agreed that basic research, designed to elucidate principles on which control measures must rest, is even more important in war-time than in peace-time, in order to avoid mistakes in the present and to lay a solid foundation for future procedures.

At a later session the need was discussed for research workers to assist in preparing an up-to-date, periodically revised manual of plant-disease control with recommendations for extension use throughout the country. Local pilot tests were held essential for successful local application of research findings and, also, for local education through their demonstration value. In a debate as to whether research, or extension workers should conduct such tests, it was claimed that in general the research man should, if possible, direct such work, but that highly effective results usually arose from cooperation between experiment station and extension service workers, including county agents.

Theoretically county agents are supposed to do a great deal of extension work in Plant Pathology. It is perfectly clear, however, that they have so many different kinds of things to do that relatively few of them are competent to play the part expected of them in disseminating information regarding plant disease control.

It was pointed out that losses from preventable diseases are still appalling. Epidemics often rage unchecked because proper control measures either are not taken at all —because they are not adequate, or because information regarding control measures had not been disseminated widely enough and at the proper time because of lack of sufficient trained personnel. One of the first and most important duties of plant pathology is prompt dissemination of information regarding the best available control measures. This responsibility cannot be discharged properly under present conditions. A survey of the situation with respect to extension plant pathologists in the country indicates that very few states have an adequate extension service. Some have no extension plant pathologist at all, and some of the most important agricultural states have but one such specialist, when two or three are needed. In only very few states can the situation with respect to extension work be considered satisfactory. Pathologists themselves are trying to do what they can by assembling and exchanging information, but the situation cannot be alleviated properly until more men are made available for this very important phase of insuring the Nation's supplies of essential materials from economic plants.

It was emphasized repeatedly that plant pathologists are finding difficulty in getting necessary things done because of the difficulty in retaining research assistants assigned to important problems. It was generally recognized that much research necessarily must be done by younger men, who are relatively free from distractions due to administrative work, public services and teaching. The national committee was instructed to study the situation and find out whether the special abilities of men subject to draft could not be used on important pathological problems, either in the places where they are now working, or in the armed forces. It was emphasized also that the depletion of the ranks of scientifically trained men constitutes a serious menace not only to present essential services to agriculture, but that it also jeopardizes the future of the discontinuance of certain basic researches that are essential in furnishing a basis for intelligent action in plant-disease control measures. Prospects are that there may be some alleviation of this situation.

Reports on "Equipment priorities" and "The activities of the fungicide committee," given at the Summer Meetings, are appended.

EQUIPMENT PRIORITIES

Throughout the current year all permitted production of spraying and dusting ma-

chinery, as well as all farm equipment, has been on the basis of a quota based on the 1940 sales of the same type machines.

The 1942 per cent quota of 1940 for the period November 1, 1941 to October 31, 1942, and effective December 31, 1941 was as follows: Power Sprayers (not incl. engine)—97; Traction Sprayers—85; Hand Sprayers (capacity less 6 gal.)—100; Sprayers (capacity 6 gal. or more)—98; Spray Pumps, power—96; Power Dusters—103; Traction Dusters—94; Hand Dusters—100; Attachments and Parts (including repairs on sprayers and dusters)—140.

March 30, 1942, the above per cent quotas were revised in the case of Traction Dusting Machines and Hand Dusters to 103 for Traction Dusters and 110 for Hand Dusters.

Priorities made it possible to secure materials for production of the above quotas only. These priorities expire June 30.

Beginning July 1, 1942 production for next season goes on a quarterly basis and each manufacturer must submit to WPB a report which includes all the materials desired for production of machines during the quarter ending September 30. How much of this will be granted is, of course, not known.

Prior to November 1 the Department of Agriculture will advise WPB what crop production they desire for 1943. On the basis of this, WPB will then decide on and authorize a per cent quota of production of sprayers and dusters for 1943 use. There is no present knowledge on what this will be.

It is probable that in September a small per cent advance production of sprayers and dusters will be permitted. Whatever the total production authorized for 1943, a good production of repair parts probably will be permitted.

Canadian manufacturers of farm equipment have already been notified that their 1943 production will be on a basis sufficient to permit the manufacture of 25% of complete machines made by them in 1940, and 150% of repairs made during that year.

REPORT OF THE FUNGICIDE SUBCOMMITTEE

The committee was instructed (1) to procure information on supply and consumption of fungicides; (2) to seek priorities, if necessary; (3) to organize nation-wide research on substitutes and dosage.

At present the War Production Board lists copper and copper scrap at the top of the list of critically short materials. They write that estimated consumption exceeds supply by half. On the list also is formaldehyde, diphenyl amine, chlorinated hydrocarbons, chlorine and phenols. Clearly, these chemicals cannot be freely used in substitutes. The War Production Board lists borax, carbon tetrachloride, casein, mercury and zinc in the group of less critically essential materials.

All fungicidal materials have been given a blanket A10 priority. Some are assigned a higher rating. Mercury is limited to 80 per cent of 1941 and prohibited for turf fungicides and wood preservation. Despite its short position, copper will be available in nearly normal quantities for 1942.

Nineteen collaborators over the country have agreed to do research on dosage and substitutes.

Most pathologists, especially those who are familiar with manufacturing problems, agree that the "best substitute for copper is copper." Therefore, the most productive research is to look into methods for "stretching supplies."

Some of the methods of stretching supplies to be investigated are: dilution, efficiency of application, particle size, and synergism with other compounds. Some of these factors have been studied in the past in relation to control. They should now be evaluated in terms of dose required for an equal level of control. Interactions of copper and sulphur have been studied. It was reported at the meeting that sulphur and cuprous oxide act synergistically not additively, so that the copper can be extended several fold.

Substitutes have been actively under study. On April 10 the committee mailed a list of 23 materials known to have fungicidal value. Since then a supplementary list of 265 organic compounds was investigated at New Haven under committee auspices. A tentative mimeographed report on these was distributed at Toledo. A more complete report will be given at the Winter Meetings at New York.